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(54) Title: ANTI-NEOVASCULATURE PREPARATIONS FOR CANCER

ED-B 29-38 specific lysis by CTL from peptide immunized HHD-A2 mice
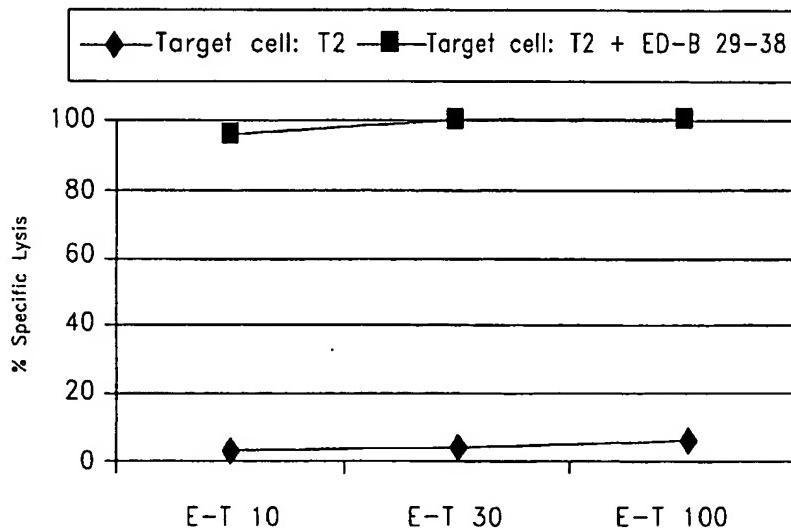
(57) Abstract: Disclosed herein are immunogenic compositions, methods of designing immunogenic compositions, methods of treatment using immunogenic compositions, methods of evaluating cell-mediated immunity resulting from immunogenic compositions, research models, and methods of making research models, all of which relate to targeting tumor vasculature.

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ANTI-NEOVASCULATURE PREPARATIONS FOR CANCER

Background of the Invention

Description of the Related Art

The treatment of cancer has remained challenging despite the advances in biomedicine. In recent years two approaches have been described showing much promise: therapeutic vaccines and anti-angiogenesis.

Therapeutic vaccines rely on the observation that cancerous tissues generally express certain antigens preferentially, collectively tumor-associated antigens (TuAA). TuAA include proteins normally expressed selectively by the tissue from which the cancer derives (differentiation antigens), proteins that are associated with a different stage of development (oncofetal and cancer-testis antigens), proteins that are created by aberrant chromosomal rearrangement, or proteins that are derived from oncogenic viruses. These TuAA, or fragments of them, are then used as immunogens in vaccines intended to stimulate cellular immunity, particularly cytotoxic T lymphocytes (CTL), capable of killing the tumor cells.

The anti-angiogenesis approach takes advantage of the need of tumors to recruit a blood supply to support their continued growth. To accomplish this, tumors secrete angiogenic factors that promote the growth of new blood vessels. The anti-angiogenesis approach aims to disrupt a tumor's supply of nutrients to cause it to die, or at least limit its growth. Attempts at this approach have sought chemotherapeutic drugs used directly against a variety of anti-angiogenic factors and angiogenesis.

Summary of the Invention

The invention disclosed herein is directed to compositions designed to stimulate cellular immune responses targeting tumor-associated neovasculature (TuNV). In one embodiment of the invention the compositions stimulate a CTL response. Such compositions may include one or more epitopes of the target antigen. One aspect of this embodiment specifically includes a housekeeping epitope, another specifically includes an immune epitope or epitope cluster, and another aspect specifically combines housekeeping and immune epitopes.

Embodiments of the invention relate to the use of prostate specific membrane antigen (PSMA) as the target antigen of the composition. Aspects of this embodiment include various epitopes derived from PSMA provided directly as polypeptide, or as a nucleic acid capable of conferring expression of the epitope. Other embodiments relate to the use of other TuNV-associated antigens.

In other embodiments of the invention, compositions are directed against both the TuNV and against TuAA expressed by the cancerous cells, by combining immunogens derived from both sources into a single formulation or method or treatment.

Preclinical evaluation of the compositions of this invention can be accomplished using adoptive transfer of immunized T cells into SCID mice bearing microvasculature formed from implanted human dermal microvascular endothelial cells (HDMEC). Preclinical evaluation can also be accomplished through the use of HLA-transgenic mice immunized with compositions comprised of epitopes conserved between mice and humans.

Embodiments of the invention relate to methods of evaluating cell-mediated immunity. The methods can include the steps of implanting vascular cells into an immunodeficient mammal; establishing an immune response in the mammal; and assaying a characteristic to determine cell-mediated immunity in the mammal. The cell-mediated immunity can be directed against a neovasculature antigen, for example. The neovasculature antigen can be preferentially expressed by tumor-associated neovasculature, for example, and in preferred embodiments can be prostate specific membrane antigen (PSMA), vascular endothelial growth factor receptor 2 (VEGFR2), and the like. The establishing step can be achieved, for example, by adoptive transfer of T-cells to the mammal, by contacting the mammal with an antigen, and the like. The cell-mediated immunity can be mediated by cytotoxic T lymphocytes. The vascular cells can be vascular endothelial cells, such as, for example, human dermal microvascular endothelial cells (HDMEC), telomerase-transformed endothelial cells, and the like. The immunodeficient mammal can be a mouse, such as for example a SCID mouse. The characterizing step can include assessing a parameter, such as for example, vessel formation, vessel destruction, vessel density, proportion of vessels carrying blood of the host mammal, and the like.

The methods can further include the step of implanting tumor cells or tumor tissue in the mouse. The characterizing step can include assessing a parameter, such as, for example, tumor presence, tumor growth, tumor size, rapidity of tumor appearance, dose of vaccine required to inhibit or prevent tumor establishment, tumor vascularization, a proportion of necrotic tissue within the tumor, and the like.

The methods can further include the steps of providing a first population of mammals and a second populations of mammals; establishing cell-mediated immunity in the first population; differentially establishing cell-mediated immunity in the second population; and comparing a result obtained from the first population of mammals to a result obtained from the second population of mammals. The cell-mediated immunity of the first population can include, for example, naïve immunity, immunity to an irrelevant epitope, and the like.

Other embodiments relate to methods of evaluating cell-mediated immunity, including immunity directed against a neovasculature antigen. The methods can include the steps of implanting or injecting MHC-transgenic tumor cells into an MHC-transgenic mammal; establishing an immune response in the mammal; and assaying a characteristic to determine cell-mediated immunity in the mammal. The MHC-transgenic mammal can be an HLA-transgenic mammal, such as, for example an HLA-A2 transgenic mammal. In preferred embodiments the mammal can be a mouse. The cell-mediated immunity can be established by vaccination, which in preferred embodiments can take place prior to, concurrent with, or subsequent to transfer of the tumor cells, for example. In preferred embodiments the cell-mediated immunity can be mediated by cytotoxic T lymphocytes. The neovasculature antigen can be preferentially expressed by tumor-associated neovasculature and can also be a tumor-associated antigen. Preferably, the antigen can be the ED-B domain of fibronectin. The characterizing step can include, for example, assessing a parameter, including tumor presence, tumor growth, tumor size, rapidity of tumor appearance, dose of vaccine required to inhibit or prevent tumor establishment, tumor vascularization, a proportion of necrotic tissue within the tumor, and the like. The methods can further include the steps of providing a first population of mammals and a second populations of mammals; establishing cell-mediated immunity in the first population; differentially establishing cell-mediated immunity in the

second population; and comparing a result obtained from the first population of mammals to a result obtained from the second population of mammals. The cell-mediated immunity of the first population can include naïve immunity, immunity to an irrelevant epitope, and the like.

Still further embodiments relate to methods of treating neoplastic disease, including the step of immunizing a mammal to induce a cellular immune response directed against an antigen differentially expressed by tumor-associated neovasculature. The differentially expressed antigen can be a protein, such as, for example prostate specific membrane antigen, vascular endothelial growth factor receptor 2 (VEGFR2), and the like. In other preferred embodiments, the antigen can be the ED-B domain of fibronectin. The immunization can be carried out, for example, with at least one peptide derived from the sequence of the protein, with a nucleic acid capable of conferring expression of the protein or peptides, and the like. The at least one peptide can include a housekeeping epitope, for example, and in preferred embodiments can be co-C-terminal with the housekeeping epitope. The methods can further include at least one additional peptide, wherein the at least one additional peptide includes an immune epitope. The methods can include an additional step wherein the mammal is treated with an anti-tumor therapy active directly against cancerous cells. The anti-tumor therapy can be immunization against a tumor-associated antigen. Preferably, the cellular immune response can include a CTL response.

Other embodiments relate to immunogenic compositions. The immunogenic compositions can include at least one immunogen corresponding to an antigen expressed by tumor-associated neovasculature, wherein the composition can induce a cellular immune response. The immunogen can be one that is not associated with a cell conspecific with the recipient. The antigen can be a protein, such as, for example prostate specific membrane antigen, vascular endothelial growth factor receptor 2 (VEGFR2), and the like. In other preferred embodiments the antigen can be the ED-B domain of fibronectin. The immunogen can include at least one peptide. The compositions can include a nucleic acid capable of conferring expression of the antigen, and wherein the antigen is a protein or a peptide. The compositions can include at least one peptide that includes a housekeeping epitope, and in preferred embodiments the at least one peptide can be co-C-terminal with the housekeeping epitope. Also, the compositions can additionally include at least one peptide that includes an immune epitope. The compositions can include at least one immunogen corresponding to a tumor-associated antigen. In preferred embodiments the cellular immune response can include a CTL response.

Embodiments relate to methods of anti-tumor vaccine design. The methods can include the steps of identifying an antigen differentially expressed by tumor-associated neovasculature; and incorporating a component of the antigen into a vaccine. The component can include, for example, a polypeptide fragment of the antigen, a nucleic acid encoding the antigen or a fragment of the antigen, and the like.

Further embodiments relate to methods of making a research model. The methods can include implanting a vascular cell and a tumor cell into an immunodeficient mammal. The tumor cell and the vascular cell can be implanted adjacent to one another. The vascular cell can be a vascular endothelial cell, such as for example HDMEC. In preferred

embodiments the vascular endothelial cell can be telomerase-transformed. The immunodeficient mammal can be a mouse, such as, for example, a SCID mouse.

Other embodiments relate to research models. The research models can include an immunodeficient mammal. The mammal can include an implanted vascular cell and an implanted tumor cell. The vascular cell and the tumor cell can be implanted adjacent to one another.

Brief Description of the Drawings

Figure 1A, B, and C show results of N-terminal pool sequencing of a T=60 min. time point aliquot of the $PSMA_{163-192}$ proteasomal digest.

Figure 2 shows binding curves for $HLA-A2:PSMA_{168-177}$ and $HLA-A2:PSMA_{288-297}$ with controls.

Figure 3 shows results of N-terminal pool sequencing of a T=60 min. time point aliquot of the $PSMA_{281-310}$ proteasomal digest.

Figure 4 shows binding curves for $HLA-A2:PSMA_{461-469}$, $HLA-A2:PSMA_{460-469}$, and $HLA-A2:PSMA_{663-671}$, with controls.

Figure 5 shows the results of a (gamma)-IFN-based ELISPOT assay detecting $PSMA_{463-471}$-reactive $HLA-A1^+$ $CD8^+$ T cells.

Figure 6 shows blocking of reactivity of the T cells used in figure 10 by anti-HLA-A1 mAb, demonstrating HLA-A1-restricted recognition.

Figure 7 shows a binding curve for $HLA-A2:PSMA_{663-671}$, with controls.

Figure 8 shows a binding curve for $HLA-A2:PSMA_{662-671}$, with controls.

Figure 9 shows epitope specific lysis by CTL from HHD-A2 mice immunized with ED-B 29-38 peptide.

Detailed Description of the Preferred Embodiment

Embodiments of the invention disclosed herein provide compositions, methods of composition or vaccine design, and methods of treatment related to the generation of a cellular immune response, preferably, a T cell response and, more preferably, a CTL response, directed against the neovasculature of tumors. Such methods and compositions are particularly useful in the treatment and prevention of cancer. Other embodiments relate to composition evaluation models.

Compositions, Composition Design, and Treatment Using the Compositions

Embodiments of the invention relate to immunogenic compositions, including vaccines, for the generation of a cellular immune response, particularly a T cell response and specifically a CTL response, directed against tumor neovasculature (TuNV). "Tumor neovasculature" is broadly meant to include any vasculature found in or around tumor masses, vasculature which supports or is necessary for tumor growth, and the like. It should be noted, and one of skill in the art will appreciate, that although the discussion herein refers generally to the tumors and tumor neovasculature, the embodiments of the present invention also can be applied to other conditions or disease states associated with inappropriate angiogenesis.

Until now the design of anti-tumor vaccines has concentrated on antigens expressed by the malignant cells themselves. However, larger tumors are complex structures and not simply a homogeneous mass of cells. All cells, particularly rapidly growing cells, need a supply of nutrients (oxygen, glucose, amino acids, etc.), as well as a means of removal of metabolic wastes, in order to remain metabolically active and intact. This is normally accomplished by the flow of blood and lymph through the various organs of the body. At a cellular level, the tissues of the body are permeated by a fine network of capillaries – tiny vessels through which nutrients and waste products can be exchanged with the surrounding cells by diffusion. Diffusion is effective over relatively short distances. The capillary beds are so extensive that generally cells are at most located only a few cells away from a capillary. If a tumor merely grew by propagation of its malignant cells, soon those cells in the interior of the mass would be unable to sustain themselves. In fact, the interiors of unvascularized tumors often contain necrotic tissue. Thus, in order to grow unchecked, tumors secrete factors that promote the in-growth of new blood vessels, namely TuNV. Since the TuNV expresses antigens differentiating it from other tissues, cancer can be treated with therapeutic compositions directed against the TuNV, instead of directly targeting the cancerous cells themselves. Suitable TuNV antigens can include those that are expressed generally in neovasculature or preferentially by TuNV, for example.

In some embodiments of the invention the compositions can include, for example, an epitopic peptide or peptides. Immune epitopes may be provided embedded in epitope clusters and protein fragments. Housekeeping epitopes can be provided with the proper C-terminus. In other embodiments of the invention the compositions can include nucleic acids capable of conferring expression of these epitopes on pAPC, for example.

In preferred embodiments, the compositions can be administered directly to the lymphatic system of a mammal being treated. This can be applied to both polypeptide and nucleic acid based compositions. Administration methods of this type, and related technologies, are disclosed in U.S. Patent Application No. 09/380,534, filed on September 1, 1999, and a Continuation-in-Part thereof, filed on February 2, 2001; U.S. Patent Application No. 09/776,232, both entitled "A METHOD OF INDUCING A CTL RESPONSE,".

In a preferred embodiment, destruction of the blood vessels in a tumor by action of a composition of the invention can eliminate *all* of the cells in a tumor. However, small tumors, including micrometastases, are typically unvascularized. Additionally, unvascularized tumors that instead apparently rely on blood flow through channels penetrating the tumor mass have been reported (Maniotis, A.J., et al. Am. J. Pathol. 155: 739-752, 1999). Thus in other embodiments, the compositions are generally effective as tumor control agents that may not eradicate all cancer cells. Accordingly, the invention provides tools for eliminating tumors, controlling tumor growth, reducing tumor burden, improving overall clinical status, and the like. In some embodiments, it can be desirable to combine these compositions with other treatments that target the cancerous cells directly. Additionally there is evidence that the vasculature in tumors can be mosaic in nature consisting of both endothelial and cancer cells (Chang, Y.S., et al. Proc. Natl. Acad. Sci. USA 97:14608-14613, 2000). Thus, in some embodiments of the invention a course of composition treatment can be followed by administration of a bio- or chemotherapeutic agent. In a particularly preferred

embodiment, treatment can include administration of a TuAA directed composition concurrent or subsequent to administration of the anti-TuNV composition.

As mentioned above, suitable TuNV antigens for the compositions can include those that are expressed generally in neovasculature or preferentially by TuNV, for example. A variety of techniques for discovery of TuAA are known in the art. Examples of these techniques include, without limitation, differential hybridization and subtractive hybridization, including use of microarrays; expression cloning; SAGE (serial analysis of gene expression); SEREX (serological identification of antigens by recombinant expression cloning); in situ RT-PCT; immunohistochemistry (as was the case for PSMA); EST analysis; variously using bulk, sectioned, and/or microdissected tissue; and the like. Utilization of these and other methods provides one of skill in the art the techniques necessary to identify genes and gene products contained within a target cell that may be used as antigens of immunogenic compositions. The techniques are applicable to TuAA discovery regardless of whether the target cell is a cancer cell or an endothelial cell. Any identified antigen can be scrutinized for epitopes, which can be used in embodiments of the invention.

The endothelial cells making up the lining of the vasculature can express housekeeping proteasomes. Thus, compositions targeting endothelial cells can be comprised of peptides, or nucleic acids conferring expression of the peptides, corresponding to the digestion products of the housekeeping proteasome (i.e. housekeeping epitopes). IFN-γ (gamma), secreted by activated cells of the immune system, can induce expression of the immunoproteasome in the target cells. Generally, the immunoproteasome is constitutive in professional antigen presenting cells (pAPC). Thus, it can be helpful to include immune epitopes or epitope clusters in CTL-inducing compositions to ensure that there are CTL able to recognize the target cell regardless of the state that the target cell is in. This can be particularly true with endothelial cells, which readily assume antigen presentation functions. These concepts are more fully explained in U.S. Patent Application Nos. 09/560,465, filed on April, 28, 2000; 10/005,905, filed on November 7, 2001; and a continuation thereof, U.S. Application No. _____ filed on December 7, 2001, attorney docket number CTLIMM.21CP1C, each of which is entitled "EPITOPE SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS,".

As discussed above, the immunogenic compositions, including in preferred embodiments, vaccines, can include TuNV antigens and epitopes, for example. The epitopes can include one or more housekeeping epitopes and/or one or more immune epitopes. Specific epitopes useful in compositions can be identified using the methods disclosed in U.S. Patent Application No. 09/561,074 entitled "METHOD OF EPITOPE DISCOVERY," filed on April 28, 2000. For example, peptide sequences that are known or predicted to bind to some MHC restriction element can be compared to fragments produced by proteasomal digestion in order to identify those that are co-C-terminal.

Examples of useful epitopes for the embodiments of the invention, including epitopes of ED-B and PSMA, are disclosed in a U.S. Provisional Patent Application No. _____, entitled "EPITOPE SEQUENCES," attorney docket number CTLIMM.027PR; filed on even date with this application, March 7, 2002, and two U.S. Provisional Patent Applications, each entitled "EPITOPE SEQUENCES;" Application No. 60/282,211, filed on April 6, 2001 and 60/337,017, filed on November 7, 2001. Each of these applications is incorporated herein by reference in its entirety.

PSMA is one example of a TuAA that can be targeted in some embodiments. PSMA is expressed in the neovasculature of most tumor types, but not by the vascular endothelium of normal tissues (Chang, S.M. et al., *Cancer Res.* 59(13):3192-8,1999; *Clin Cancer Res.* 10:2674-81, 1999). PSMA is a membrane antigen, and as such, it may be possible to attack PSMA-expressing TuNV with monoclonal antibody (mAb). However, the effectiveness of mAb in the treatment of cancer has proved to be more difficult than initially anticipated. Moreover, as other antigens are discovered to be associated with the TuNV, it is likely that many of them will prove not to be expressed at the vasculature surface, making them inaccessible to mAb attack.

T cells, particularly CTL, on the other hand, survey the expression of internal components of the cell through the process of major histocompatability complex (MHC)—restricted antigen presentation. The parameters for determining the effectiveness of T cell-activating vaccines and compositions against self-antigens are subtle. Some of the critical features and parameters relating to appropriate epitope selection are disclosed in U.S. Patent Application No. 09/560,465 entitled "EPITOPE SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS," filed on April 28, 2001; U.S. Patent Application No. 09/561,074 entitled "METHOD OF EPITOPE DISCOVERY," filed on April 28, 2001; and U.S. Patent Application No. 09/561,571 entitled "EPITOPE CLUSTERS," filed on April 28, 2001. Features of DNA vaccine design promoting epitope synchronization are disclosed in U.S. Patent Application No. 09/561,572 entitled "EXPRESSION VECTORS ENCODING EPITOPES OF TARGET-ASSOCIATED ANTIGENS," filed on April 28, 2001 and U.S. Provisional Application No. 60/336,968 entitled "EXPRESSION VECTORS ENCODING EPITOPES OF TARGET-ASSOCIATED ANTIGENS' AND METHODS FOR THEIR DESIGN," filed on November 7, 2001. Particularly effective means of vaccine delivery are described in U.S. Patent Application No. 09/380,534, filed on September 1, 1999, and a Continuation-in-Part thereof, U.S. Patent Application No. 09/776,232, filed on February 2, 2001, both entitled "A METHOD OF INDUCING A CTL RESPONSE." Each of the above-mentioned references is incorporated herein by reference in its entirety.

Another example of a TuNV antigen that can be used in embodiments is fibronectin, preferably the ED-B domain. Fibronectin is subject to developmentally regulated alternative splicing, with the ED-B domain being encoded by a single exon that is used primarily in oncofetal tissues. Matsuura, H. and S. Hakomori *Proc. Natl. Acad. Sci. USA* 82:6517-6521, 1985; Carnemolla, B. et al. *J. Cell Biol.* 108:1139-1148, 1989; Loridon-Rosa, B. et al. *Cancer Res.*50:1608-1612, 1990; Nicolo, G. et al. *Cell Differ. Dev.* 32:401-408, 1990; Borsi, L. et al. *Exp. Cell Res.* 199:98-105, 1992; Oyama, F. et al. *Cancer Res.* 53:2005-2011, 1993; Mandel, U. et al. *APMIS* 102:695-702, 1994; Farnoud, M.R. et al. *Int. J. Cancer* 61:27-34, 1995; Pujuguet, P. et al. Am. J. Pathol. 148:579-592, 1996; Gabler, U. et al. *Heart* 75:358-362, 1996; Chevalier, X. *Br. J. Rheumatol.* 35:407-415, 1996; Midulla, M. *Cancer Res.* 60:164-169, 2000. '

The ED-B domain is also expressed in fibronectin of the neovasculature Kaczmarek, J. et al. *Int. J. Cancer* 59:11-16, 1994; Castellani, P. et al. *Int. J. Cancer* 59:612-618, 1994; Neri, D. et al. *Nat. Biotech.* 15:1271-1275, 1997; Karelina, T.V. and A.Z. Eisen *Cancer Detect. Prev.* 22:438-444, 1998; Tarli, L. et al. *Blood* 94:192-198, 1999; Castellani, P. et al. *Acta Neurochir. (Wien)* 142:277-282, 2000. As an oncofetal domain, the ED-B domain is

commonly found in the fibronectin expressed by neoplastic cells, in addition to being expressed by the TuNV. Therefore, CTL-inducing compositions targeting the ED-B domain can exhibit two mechanisms of action: direct lysis of tumor cells, and disruption of tumor blood supply through destruction of the TuNV.

5   It should be noted that expression of the fibronectin ED-B domain has been reported in both tumor-associated and normal neovasculature (Castellani, P. et al. *Int. J. Cancer* 59:612-618, 1994). Thus, compositions based on it, or similarly expressed antigens, can be effective against other conditions associated with inappropriate angiogenesis. Further, as CTL activity can decay rapidly after withdrawal of the composition, interference with normal angiogenesis can be minimal.

       Other targets for the immunogenic compositions include growth factor receptors, including those associated
10   with vascular cells. One such example is the vascular endothelial growth factor receptor 2 (VEGFR2). U.S. Patent No. 6,342,221 includes discussion of VEGF and VEGFR2. One of skill in the art will appreciate that any other antigen or protein associated with vascular cells can be a target for the immunogenic compositions, including those that are presently known and those yet to be identified.

Animal Models, Methods of Making the Models, and Composition Evaluation

15   Compositions designed based upon the preceding considerations are effective against the various targets. However, additional evaluation can be easily performed at any time, but preferably in a pre-clinical setting. For example, such evaluation can be used in order to further aid in composition design. Other embodiments of the invention relate to methods of evaluating the immunogenic compositions. The compositions of the present invention can be easily evaluated by one of skill in the art using animal models for composition evaluation. For example,
20   following the routine procedures below, one of skill in the art can evaluate TuNV compositions quickly and efficiently. Thus, using the models or guidance described herein, one of skill in the art can evaluate any TuNV composition for any TuNV antigen with little or no experimentation. Further embodiments relate to methods of making the animal research models. Other embodiments relate to the research model animals. These embodiments are set forth more fully below.

Xenotransplanted Human Vasculature-Based Model

25   Some embodiments relate to a model system for studying the mechanisms of human microvessel formation. For example, in some embodiments, the model system can be used for preclinical evaluation of compositions. The model involves the subcutaneous implantation of telomerase-transformed human dermal microvascular endothelial cells (HDMEC) mixed with MATRIGEL (Becton Dickinson) into SCID mice. Subcutaneous implantation of telomerase-transformed HCMEC is described in Yang, J. et al. Nature Biotech 19:219-224, 2001. T cells activated by the
30   compositions of this invention can be adoptively transferred, for example, into such implanted mice, and the ability of the T cells to destroy, or prevent the formation of, such human microvessels can be assessed. In other embodiments, the mouse can be directly vaccinated and evaluated. Also, in still further embodiments, the model system can be further adapted for testing compositions effective in non-human species by substituting DMEC from other species and species-matched telomerase, and by using analogous reagents to those described below for the human system.

The MHC-restriction elements presenting the epitopes of the composition being tested, preferably, are shared by the HDMEC line implanted into the mice. The T cells can be derived from *in vitro* immunization of human T cells, or by immunization of HLA-transgenic mice (procedures for which are well known in the art and examples of which are provided in the above incorporated patent applications). Use of T cells generated in HLA-transgeneic mice allows matching of genetic backgrounds between the adoptively transferred T cells and the host, thereby reducing the possibility of allogeneic or xenogeneic reactions that might complicate interpretation of the results. However, depending on the mouse strains available, this might require cross-breeding to get the HLA-transgene and SCID phenotype on the same genetic background. In the alternative, the donor T cells (human or murine) can be subjected to one or more rounds of *in vitro* stimulation to enrich for the desired population or establish a clone, and thereby similarly avoid undesired reactivities.

Techniques for *in vitro* immunization are know in the art, for example, Stauss et al., *Proc. Natl. Acad. Sci. USA* 89:7871-7875, 1992; Salgaller et al. *Cancer Res.* 55:4972-4979, 1995; Tsai et al., *J. Immunol.* 158:1796-1802, 1997; and Chung et al., *J. Immunother.* 22:279-287, 1999. Once generated, whether *in vivo* or *in vitro*, sufficient numbers of such T cells can be obtained by expansion *in vitro* through stimulation with the compositions of this invention and/or cytokines (see for example Kurokawa, T. et al., *Int. J. Cancer* 91:749-746, 2001) or other mitogens. These T cells can constitute a clone or a polyclonal population recognizing one or more epitopes. In preferred embodiments, on the order of 10^5 to 10^8 cells are transferred for adoptive transfer experiments in mice. (See for example Drobyski, W.R. et al. *Blood* 97:2506-2513, 2001; Seeley B.M. et al. *Otolaryngol. Head Neck Surg.* 124:436-441, 2001; Kanwar, J.R. et al. *Cancer Res.* 61:1948-1956, 2001). Clones and otherwise more enriched populations generally require the transfer of fewer cells.

Transfer of the T cells can take place shortly before, concurrent with, or subsequent to implantation or establishment of the HDMEC. Parameters that can be assessed to evaluate effectiveness of the compositions include vessel formation, changes in vessel density, and ability to carry mouse blood (as described in Yang et al.), and the like. Assessment can be carried out as early as one week, and at least as long as 6 weeks, after implantation of telomerase-transformed HDMEC, preferably after 2 weeks; and from a day to more than 6 weeks after T cell transfer, preferably after 1 to 3 weeks. Generally, assessment can include comparison of mice receiving T cells reactive with the target antigen with mice receiving naïve (including sham-immunized), or irrelevant epitope-reactive T cells.

Relevant antigens can be expressed generally in neovasculature or preferentially by TuNV. Expression can be confirmed by a variety of techniques known in the art, including immunohistochemistry and RT-PCR. For example, tumor cells can be implanted along with the HDMEC. This can result in inducing expression of antigens preferentially expressed by TuNV. In one example, this can be accomplished by implanting a block of tumor tissue adjacent to the HDMEC-containing MATRIGEL implant, injecting tumor cells at the site of the implant, implanting tumor cell-containing MATRIGEL adjacent to the HDMEC-containing MATRIGEL implant, incorporating both tumor cells and HDMEC into the same MATRIGEL implant or by any other suitable method. As discussed above, in some embodiments, tumor cells can

be implanted along with vascular cells. The animals so made, can be used as research models. Additional variations will be apparent to one of skill in the art.

HLA-transgenic Animal Model

For antigens that are conserved, in sequence and/or expression profile, between human and the model species, HLA-transgenic strains allow another approach, namely vaccination of the model animal to combat a syngeneic tumor. The ED-B domain of fibronectin provides such an opportunity, as it is a marker of angiogenesis and has identical amino acid sequence in both humans and mice (Nilsson, F. et al. *Cancer Res.* 61:711-716, 2001). Moreover, spontaneous tumor tissue from a strain of HLA-A2 transgenic mice has been isolated and propagated. Epitope discovery and selection, and composition design and delivery for CTL inducing compositions are discussed above.

The tumor cell line, M1, is derived from a spontaneous salivary glandular cystadenocarcinoma. The M1 tumor cell line and methods of using the same is disclosed in U.S. Provisional Application No. _____, attorney docket number CTLIMM.028PR, filed on even date with this application, March 7, 2001, entitled "AN HLA-TRANSGENIC MURINE TUMOR CELL LINE." The tumor cell line, can arise in individuals of the HHD-A2 transgenic mouse strain of S. Pascolo et al. (J. Exp. Med. 185:2043-2051, 1997). These mice express a single monochain class I molecule comprising human ß (beta)2-microglobulin, and α1 (alpha-1), and α2 (alpha-2) domains of HLA-A2.1 with the balance of the molecule derived from the murine class I molecule H2D^b. Blocks of tumor can be transplanted into new individuals where the tumor will re-grow, commonly within 1-3 weeks, with 3 mm blocks growing to 3 cm. Alternatively, tumor tissue can be disaggregated and the tumor cells grown *in vitro*. Upon harvest, the tumor cells can be injected subcutaneously into the neck or abdomen (2.5x10^6 cells for 1-3 successive days), to result in a visible tumor in approximately 5-12 weeks for early passage cells. After the cells have become better adapted to growth *in vitro*, single injections of 1x10^6 to 1x10^7 cells lead to visible tumor in ten days. Generally, the initial tumor consistently occurs in the vicinity of the salivary glands, but secondary tumors can also occur in a variety of locations, including kidney, lung, liver, and abdominal muscle.

To evaluate the efficacy of a composition, it can be administered before, concurrent with, or subsequent to establishment of the tumor, depending on the expected mechanism of the composition. For therapeutic compositions intended to be used with some sort of debulking technique (e.g. surgery), concurrent administration can be appropriate. The better established the tumor is before treatment begins, the more stringent the test.

Both animal evaluation models have been described for the testing of human compositions. However, application to veterinary compositions is analogous, requiring only the substitution of species-matched endothelial cells, MHC, TuAA, etc.

The following examples are intended for illustration purposes only, and should not be construed as limiting the scope of the invention in any way.

EXAMPLES

Example 1.

A preclinical study was carried out using the already identified antigens PSMA and ED-B disclosed herein. The results of the study revealed excellent candidate epitopes. See table 9 below.

Example 1.1

Cluster Analysis (PSMA$_{163-192}$)

A peptide, AFSPQGMPEGDLVYVNYARTEDFFKLERDM, PSMA$_{163-192}$, (SEQ ID NO. 3), containing an A1 epitope cluster from prostate specific membrane antigen, PSMA$_{168-190}$ (SEQ ID NO. 4) was synthesized using standard solid-phase F-moc chemistry on a 433A ABI Peptide synthesizer. After side chain deprotection and cleavage from the resin, peptide first dissolved in formic acid and then diluted into 30% Acetic acid, was run on a reverse-phase preparative HPLC C4 column at following conditions: linear AB gradient (5% B/min) at a flow rate of 4 ml/min, where eluent A is 0.1% aqueous TFA and eluent B is 0.1% TFA in acetonitrile. A fraction at time 16.642 min containing the expected peptide, as judged by mass spectrometry, was pooled and lyophilized. The peptide was then subjected to proteasome digestion and mass spectrum analysis essentially as described above. Prominent peaks from the mass spectra are summarized in Table 1.

Table 1. PSMA$_{163-192}$ Mass Peak Identification.

PEPTIDE	SEQUENCE	CALCULATED MASS (MH⁺)
163-177	AFSPQGMPEGDLVYV	1610.0
178-189	NYARTEDFFKLE	1533.68
170-189	PEGDLVYVNYARTEDFFKLE	2406.66
178-191	NYARTEDFFKLERD	1804.95
170-191	PEGDLVYVNYARTEDFFKLERD	2677.93
178-192	NYARTEDFFKLERDM	1936.17
163-176	AFSPQGMPEGDLVY	1511.70
177-192	VNYARTEDFFKLERDM	2035.30
163-179	AFSPQGMPEGDLVYVNY	1888.12
180-192	ARTEDFFKLERDM	1658.89
163-183	AFSPQGMPEGDLVYVNYARTE	2345.61
184-192	DFFKLERDM	1201.40
176-192	YVNYARTEDFFKLERDM	2198.48
167-185	QGMPEGDLVYVNYARTEDF	2205.41
178-186	NYARTEDFF	1163.22

Boldface sequences correspond to peptides predicted to bind to MHC, see Table 2.

N-terminal Pool Sequence Analysis

One aliquot at one hour of the proteasomal digestion was subjected to N-terminal amino acid sequence analysis by an ABI 473A Protein Sequencer (Applied Biosystems, Foster City, CA). Determination of the sites and efficiencies of cleavage was based on consideration of the sequence cycle, the repetitive yield of the protein sequencer, and the relative yields of amino acids unique in the analyzed sequence. That is if the unique (in the analyzed sequence) residue X appears only in the nth cycle a cleavage site exists n-1 residues before it in the N-terminal direction. In addition to helping resolve any ambiguity in the assignment of mass to sequences, these data also provide a more reliable indication of the relative yield of the various fragments than does mass spectrometry.

For $PSMA_{163-192}$ (SEQ ID NO. 3) this pool sequencing supports a single major cleavage site after V_{177} and several minor cleavage sites, particularly one after Y_{179}. Reviewing the results presented in figures 1A-C reveals the following:

S at the 3rd cycle indicating presence of the N-terminus of the substrate.

Q at the 5th cycle indicating presence of the N-terminus of the substrate.

N at the 1st cycle indicating cleavage after V_{177}.

N at the 3rd cycle indicating cleavage after V_{175}. Note the fragment 176-192 in Table 1.

T at the 5th cycle indicating cleavage after V_{177}.

T at the 1st –3rd cycles, indicating increasingly common cleavages after R_{181}, A_{180} and Y_{179}. Only the last of these correspond to peaks detected by mass spectrometry; 163-179 and 180-192, see Table 1. The absence of the others can indicate that they are on fragments smaller than were examined in the mass spectrum.

K at the 4th, 8th, and 10th cycles indicating cleavages after E_{183}, Y_{179}, and V_{177}, respectively, all of which correspond to fragments observed by mass spectroscopy. See Table 1.

A at the 1st and 3rd cycles indicating presence of the N-terminus of the substrate and cleavage after V_{177}, respectively.

P at the 4th and 8th cycles indicating presence of the N-terminus of the substrate.

G at the 6th and 10th cycles indicating presence of the N-terminus of the substrate.

M at the 7th cycle indicating presence of the N-terminus of the substrate and/or cleavage after F_{185}.

M at the 15th cycle indicating cleavage after V_{177}.

The 1st cycle can indicate cleavage after D_{181}, see Table 1.

R at the 4th and 13th cycle indicating cleavage after V_{177}.

R at the 2nd and 11th cycle indicating cleavage after Y_{179}.

V at the 2nd, 6th, and 13th cycle indicating cleavage after V_{175}, M_{169} and presence of the N-terminus of the substrate, respectively. Note fragments beginning at 176 and 170 in Table 1.

Y at the 1st, 2nd, and 14th cycles indicating cleavage after V_{175}, V_{177}, and presence of the N-terminus of the substrate, respectively.

L at the11th and 12th cycles indicating cleavage after V$_{177}$, and presence of the N-terminus of the substrate, respectively, is the interpretation most consistent with the other data. Comparing to the mass spectrometry results we see that L at the 2nd, 5th, and 9th cycles is consistent with cleavage after F$_{188}$, E$_{183}$ or M$_{169}$, and Y$_{179}$, respectively. See Table 1.

Epitope Identification

Fragments co-C-terminal with 8-10 amino acid long sequences predicted to bind HLA by the SYFPEITHI or NIH algorithms were chosen for further analysis. The digestion and prediction steps of the procedure can be usefully practiced in any order. Although the substrate peptide used in proteasomal digest described here was specifically designed to include a predicted HLA-A1 binding sequence, the actual products of digestion can be checked after the fact for actual or predicted binding to other MHC molecules. Selected results are shown in Table 2.

Table 2. Predicted HLA binding by proteasomally generated fragments

I. SEQ ID NO	II. PEPTIDE	HLA	SYFPEITHI	NIH
5 & (6)	(G)MPEGDLVYV	A*0201	17 (27)	(2605)
		B*0702	20	<5
		B*5101	22	314
7 & (8)	(Q)GMPEGDLVY	A1	24 (26)	<5
		A3	16 (18)	36
		B*2705	17	25
9	MPEGDLVY	B*5101	15	NP†
10 & (11)	(P)EGDLVYVNY	A1	27 (15)	12
		A26	23 (17)	NP
12	LVYVNYARTE	A3	21	<5
13 & (14)	(Y)VNYARTEDF	A26	(20)	NP
		B*08	15	<5
		B*2705	· 12	50
15	NYARTEDFF	A24	NP†	100
		Cw*0401	NP	120
16	YARTEDFF	B*08	16	<5
17	RTEDFFKLE	A1	21	<5
		A26	15	NP

†No prediction

HLA-A*0201 binding assay:

Binding of the candidate epitope PSMA$_{168-177}$, GMPEGDLVYV, (SEQ ID NO. 6) to HLA-A2.1 was assayed using a modification of the method of Stauss et al., (Proc Natl Acad Sci USA 89(17):7871-5 (1992)). Specifically, T2 cells, which express empty or unstable MHC molecules on their surface, were washed twice with Iscove's modified Dulbecco's medium (IMDM) and cultured overnight in serum-free AIM-V medium (Life Technologies, Inc., Rockville, MD)

supplemented with human ß2-microglobulin at 3µg/ml (Sigma, St. Louis, MO) and added peptide, at 800, 400, 200, 100, 50, 25, 12.5, and 6.25 µg/ml.in a 96-well flat-bottom plate at 3x10⁵ cells/200 µl/well. Peptide was mixed with the cells by repipeting before distributing to the plate (alternatively peptide can be added to individual wells), and the plate was rocked gently for 2 minutes. Incubation was in a 5% CO_2 incubator at 37°C. The next day the unbound

5 · peptide was removed by washing twice with serum free RPMI medium and a saturating amount of anti-class I HLA monoclonal antibody, fluorescein isothiocyanate (FITC)-conjugated anti-HLA A2, A28 (One Lambda, Canoga Park, CA) was added. After incubation for 30 minutes at 4°C, cells were washed 3 times with PBS supplemented with 0.5% BSA, 0.05%(w/v) sodium azide, pH 7.4-7.6 (staining buffer). (Alternatively W6/32 (Sigma) can be used as the anti-class I HLA monoclonal antibody the cells washed with staining buffer and then incubated with fluorescein

10 isothiocyanate (FITC)-conjugated goat F(ab') antimouse-IgG (Sigma) for 30 min at 4°C and washed 3 times as before.) The cells were resuspended in 0.5 ml staining buffer. The analysis of surface HLA-A2.1 molecules stabilized by peptide binding was performed by flow cytometry using a FACScan (Becton Dickinson, San Jose, CA). If flow cytometry is not to be performed immediately the cells can be fixed by adding a quarter volume of 2% paraformaldehyde and storing in the dark at 4 C.

15   As seen in figure 2, this epitope exhibits significant binding at even lower concentrations than the positive control peptides. The Melan-A peptide used as a control in this assay (and throughout this disclosure), ELAGIGILTV (SEQ ID NO: 106), is actually a variant of the natural sequence (EAAGIGILTV; SEQ ID NO: 107)) and exhibits a high affinity in this assay. The known A2.1 binder FLPSDYFPSV (HBV$_{18-27}$; SEQ ID NO: 107) was also used as a positive control. An HLA-B44 binding peptide, AEMGKYSFY (SEQ ID NO: 109), was used as a negative control. The

20 fluorescence obtained from the negative control was similar to the signal obtained when no peptide was used in the assay. Positive and negative control peptides were chosen from Table 18.3.1 in *Current Protocols in Immunology* p. 18.3.2, John Wiley and Sons, New York, 1998.

Example 1.2

Cluster Analysis (PSMA$_{281-310}$).

25   Another peptide, RGIAEAVGLPSIPVHPIGYYDAQKLLEKMG, PSMA$_{281-310}$, (SEQ ID NO. 18), containing an A1 epitope cluster from prostate specific membrane antigen, PSMA$_{283-307}$ (SEQ ID NO. 19), was synthesized using standard solid-phase F-moc chemistry on a 433A ABI Peptide synthesizer. After side chain deprotection and cleavage from the resin, peptide in ddH2O was run on a reverse-phase preparative HPLC C18 column at following conditions: linear AB gradient (5% B/min) at a flow rate of 4 ml/min, where eluent A is 0.1% aqueous TFA and eluent B is 0.1%

30 TFA in acetonitrile. A fraction at time 17.061 min containing the expected peptide as judged by mass spectrometry, was pooled and lyophilized. The peptide was then subjected to proteasome digestion and mass spectrum analysis essentially as described above. Prominent peaks from the mass spectra are summarized in Table 3.

Table 3. PSMA$_{281-310}$ Mass Peak Identification.

PEPTIDE	SEQUENCE	CALCULATED MASS (MH⁺)
281-297	RGIAEAVGLPSIPVHPI*	1727.07
286-297	AVGLPSIPVHPI**	1200.46
287-297	VGLPSIPVHPI	1129.38
288-297	GLPSIPVHPI†	1030.25
298-310	GYYDAQKLLEKMG‡	1516.5
298-305	GYYDAQKL§	958.05
281-305	RGIAEAVGLPSIPVHPIGYYDAQKL	2666.12
281-307	RGIAEAVGLPSIPVHPIGYYDAQKLLE	2908.39
286-307	AVGLPSIPVHPIGYYDAQKLLE¶	2381.78
287-307	VGLPSIPVHPIGYYDAQKLLE	2310.70
288-307	GLPSIPVHPIGYYDAQKLLE#	2211.57
281-299	RGIAEAVGLPSIPVHPIGY	1947
286-299	AVGLPSIPVHPIGY	1420.69
287-299	VGLPSIPVHPIGY	1349.61
288-299	GLPSIPVHPIGY	1250.48
287-310	VGLPSIPVHPIGYYDAQKLLEKMG	2627.14
288-310	GLPSIPVHPIGYYDAQKLLEKMG	2528.01

Boldface sequences correspond to peptides predicted to bind to MHC, see Table 4.
*By mass alone this peak could also have been 296-310 or 288-303.
**By mass alone this peak could also have been 298-307. Combination of HPLC and mass spectrometry show that at
some later time points this peak is a mixture of both species.
† By mass alone this peak could also have been 289-298.
  By mass alone this peak could also have been 281-295 or 294-306.
§ By mass alone this peak could also have been 297-303.
¶ By mass alone this peak could also have been 285-306.
# By mass alone this peak could also have been 288-303.
None of these alternate assignments are supported N-terminal pool sequence analysis.

N-terminal Pool Sequence Analysis

One aliquot at one hour of the proteasomal digestion (see Example 3 part 3 above) was subjected to N-
terminal amino acid sequence analysis by an ABI 473A Protein Sequencer (Applied Biosystems, Foster City, CA).
Determination of the sites and efficiencies of cleavage was based on consideration of the sequence cycle, the
repetitive yield of the protein sequencer, and the relative yields of amino acids unique in the analyzed sequence. That
is if the unique (in the analyzed sequence) residue X appears only in the nth cycle a cleavage site exists n-1 residues
before it in the N-terminal direction. In addition to helping resolve any ambiguity in the assignment of mass to
sequences, these data also provide a more reliable indication of the relative yield of the various fragments than does
mass spectrometry.

For PSMA$_{281\text{-}310}$ (SEQ ID NO. 18) this pool sequencing supports two major cleavage sites after V$_{287}$ and I$_{297}$ among other minor cleavage sites. Reviewing the results presented in Fig. 3 reveals the following:

S at the 4th and 11th cycles indicating cleavage after V$_{287}$ and presence of the N-terminus of the substrate, respectively.

H at the 8th cycle indicating cleavage after V$_{287}$. The lack of decay in peak height at positions 9 and 10 versus the drop in height present going from 10 to 11 can suggest cleavage after A$_{286}$ and E$_{285}$ as well, rather than the peaks representing latency in the sequencing reaction.

D at the 2nd, 4th, and 7th cycles indicating cleavages after Y$_{299}$, I$_{297}$, and V$_{294}$, respectively. This last cleavage is not observed in any of the fragments in Table 4 or in the alternate assignments in the notes below.

Q at the 6th cycle indicating cleavage after I$_{297}$.

M at the 10th and 12th cycle indicating cleavages after Y$_{299}$ and I$_{297}$, respectively.

Epitope Identification

Fragments co-C-terminal with 8-10 amino acid long sequences predicted to bind HLA by the SYFPEITHI or NIH algorithms were chosen for further study. The digestion and prediction steps of the procedure can be usefully practiced in any order. Although the substrate peptide used in proteasomal digest described here was specifically designed to include a predicted HLA-A1 binding sequence, the actual products of digestion can be checked after the fact for actual or predicted binding to other MHC molecules. Selected results are shown in Table 4.

Table 4.
Predicted HLA binding by proteasomally generated fragments: PSMA$_{281\text{-}310}$

III. SEQ ID NO.	IV. PEPTIDE	HLA	SYFPEITHI	NIH
20 & (21)	(G)LPSIPVHPI	A*0201	16 (24)	(24)
		B*0702/B7	23	12
		B*5101	24	572
		Cw*0401	NP†	20
22 & (23)	(P)IGYYDAQKL	A*0201	(16)	<5
		A26	(20)	NP
		B*2705	16	25
		B*2709	15	NP
		B*5101	21	57
		Cw*0301	NP	24
24 & (25)	(P)SIPVHPIGY	A1	21 (27)	<5
		A26	22	NP
		A3	16	<5
26	IPVHPIGY	B*5101	16	NP
27	YYDAQKLLE	A1	22	<5

†No prediction

As seen in Table 4, N-terminal addition of authentic sequence to epitopes can often generate still useful, even better epitopes, for the same or different MHC restriction elements. Note for example the pairing of (G)LPSIPVHPI with HLA-A*0201, where the 10-mer can be used as a vaccine useful with several MHC types by relying on N-terminal trimming to create the epitopes for HLA-B7, -B*5101, and Cw*0401.

HLA-A*0201 binding assay:

HLA-A*0201 binding studies were preformed with PSMA$_{288-297}$, GLPSIPVHPI, (SEQ ID NO. 21) essentially as described in Example 1.1 above. As seen in figure 2, this epitope exhibits significant binding at even lower concentrations than the positive control peptides.

Example 1.3

Cluster Analysis (PSMA$_{454-481}$).

Another peptide, SSIEGNYTLRVDCTPLMYSLVHLTKEL, PSMA$_{454-481}$, (SEQ ID NO. 28) containing an epitope cluster from prostate specific membrane antigen, was synthesized by MPS (purity >95%) and subjected to proteasome digestion and mass spectrum analysis as described above. Prominent peaks from the mass spectra are summarized in Table 5.

Table 5. PSMA$_{454-481}$ Mass Peak Identification.

MS PEAK (measured)	PEPTIDE	SEQUENCE	CALCULATED MASS (MH⁺)
1238.5	454-464	SSIEGNYTLRV	1239.78
1768.38±0.60	454-469	SSIEGNYTLRVDCTPL	1768.99
1899.8	454-470	SSIEGNYTLRVDCTPLM	1900.19
1097.63±0.91	463-471	**RVDCTPLMY**	1098.32
2062.87±0.68	454-471*	SSIEGNYTLRVDCTPLMY	2063.36
1153	472-481**	**SLVHNLTKEL**	1154.36
1449.93±1.79	470-481	MYSLVHNLTKEL	1448.73

Boldface sequence correspond to peptides predicted to bind to MHC, see Table 6.
* On the basis of mass alone this peak could equally well be assigned to the peptide 455-472 however proteasomal removal of just the N-terminal amino acid is considered unlikely. If the issue were important it could be resolved by N-terminal sequencing.
**On the basis of mass this fragment might also represent 455-464.
Epitope Identification

Fragments co-C-terminal with 8-10 amino acid long sequences predicted to bind HLA by the SYFPEITHI or NIH algorithms were chosen for further study. The digestion and prediction steps of the procedure can be usefully practiced in any order. Although the substrate peptide used in proteasomal digest described here was specifically designed to include predicted HLA-A2.1 binding sequences, the actual products of digestion can be checked after the fact for actual or predicted binding to other MHC molecules. Selected results are shown in Table 6.

Table 6. Predicted HLA binding by proteasomally generated fragments

V. SEQ ID NO	VI. PEPTIDE	HLA	SYFPEITHI	NIH
29 & (30)	(S)IEGNYTLRV	A1	(19)	<5
31	EGNYTLRV	A*0201	16 (22)	<5
		B*5101	15	NP†
32 & (33)	(Y)TLRVDCTPL	A*0201	20 (18)	(5)
		A26	16 (18)	NP
		B7	14	40
		B8	23	<5
		B*2705	12	30
		Cw*0301	NP	(30)
34	LRVDCTPLM	B*2705	20	600
		B*2709	20	NP
35 & (36)	(L)RVDCTPLMY	A1	32 (22)	125 (13.5)
		A3	25	<5
		A26	22	NP
		B*2702	NP	(200)
		B*2705	13 (NP)	(1000)

†No prediction

As seen in Table 6, N-terminal addition of authentic sequence to epitopes can often generate still useful, even better epitopes, for the same or different MHC restriction elements. Note for example the pairing of (L)RVDCTPLMY (SEQ ID NOS 35 and (36)) with HLA-B*2702/5, where the 10-mer has substantial predicted halftimes of dissociation and the co-C-terminal 9-mer does not. Also note the case of SIEGNYTLRV (SEQ ID NO 30) a predicted HLA-A*0201 epitope which can be used as a vaccine useful with HLA-B*5101 by relying on N-terminal trimming to create the epitope.

HLA-A*0201 binding assay

HLA-A*0201 binding studies were preformed, essentially as described in Example 1.1 above, with $PSMA_{460-469}$, YTLRVDCTPL, (SEQ ID NO.33). As seen in figure 4, this epitope was found to bind HLA-A2.1 to a similar extent as the known A2.1 binder FLPSDYFPSV (HBV_{18-27}; SEQ ID NO: 108) used as a positive control. Additionally, $PSMA_{461-469}$, (SEQ ID NO. 32) binds nearly as well.

ELISPOT analysis: $\underline{PSMA_{463-471}}$ (SEQ ID NO. 35)

The wells of a nitrocellulose-backed microtiter plate were coated with capture antibody by incubating overnight at 4°C using 50 µl/well of 4µg/ml murine anti-human -IFN monoclonal antibody in coating buffer (35 mM sodium bicarbonate, 15 mM sodium carbonate, pH 9.5). Unbound antibody was removed by washing 4 times 5 min. with PBS. Unbound sites on the membrane then were blocked by adding 200µl/well of RPMI medium with 10% serum and incubating 1 hr. at room temperature. Antigen stimulated $CD8^+$ T cells, in 1:3 serial dilutions, were seeded into the wells of the microtiter plate using 100µl/well, starting at 2×10^5 cells/well. (Prior antigen stimulation was

essentially as described in Scheibenbogen, C. et al. *Int. J. Cancer* 71:932-936, 1997; which is incorporated herein by reference in its entirety.) PSMA$_{462-471}$ (SEQ ID NO. 36) was added to a final concentration of 10μg/ml and IL-2 to 100 U/ml and the cells cultured at 37°C in a 5% CO$_2$, water-saturated atmosphere for 40 hrs. Following this incubation the plates were washed with 6 times 200 μl/well of PBS containing 0.05% Tween-20 (PBS-Tween). Detection antibody,

5   50μl/well of 2g/ml biotinylated murine anti-human -IFN monoclonal antibody in PBS + 10% fetal calf serum, was added and the plate incubated at room temperature for 2 hrs. Unbound detection antibody was removed by washing with 4 times 200 μl of PBS-Tween. 100μl of avidin-conjugated horseradish peroxidase (Pharmingen, San Diego, CA) was added to each well and incubated at room temperature for 1 hr. Unbound enzyme was removed by washing with 6 times 200 μl of PBS-Tween. Substrate was prepared by dissolving a 20 mg tablet of 3-amino 9-ethylcoarbasole in 2.5

10   ml of N, N-dimethylformamide and adding that solution to 47.5 ml of 0.05 M phosphate-citrate buffer (pH 5.0). 25 μl of 30% H$_2$O$_2$ was added to the substrate solution immediately before distributing substrate at100 μl/well and incubating the plate at room temperature. After color development (generally 15-30 min.), the reaction was stopped by washing the plate with water. The plate was air dried and the spots counted using a stereomicroscope.

Figure 5 shows the detection of PSMA$_{463-471}$ (SEQ ID NO. 35)-reactive HLA-A1$^+$ CD8$^+$ T cells previously

15   generated in cultures of HLA-A1$^+$ CD8$^+$ T cells with autologous dendritic cells plus the peptide. No reactivity is detected from cultures without peptide (data not shown). In this case it can be seen that the peptide reactive T cells are present in the culture at a frequency between 1 in 2.2x10^4 and 1 in 6.7x10^4. That this is truly an HLA-A1-restricted response is demonstrated by the ability of anti-HLA-A1 monoclonal antibody to block -IFN production; see figure 6.

20   **Example 1.4**

Cluster Analysis (PSMA$_{653-687}$).

Another peptide, FDKSNPIVLRMMNDQLMFLERAFIDPLGLPDRPFY PSMA$_{653-687}$, (SEQ ID NO. 37) containing an A2 epitope cluster from prostate specific membrane antigen, PSMA$_{660-681}$ (SEQ ID NO. 38), was synthesized by MPS (purity > 95%) and subjected to proteasome digestion and mass spectrum analysis as described above. Prominent

25   peaks from the mass spectra are summarized in Table 7.

Table 7. PSMA$_{653-687}$ **Mass Peak Identification.**

MS PEAK (measured)	PEPTIDE	SEQUENCE	CALCULATED MASS (MH⁺)
906.17±0.65	681-687**	LPDRPFY	908.05
1287.73±0.76	677-687**	DPLGLPDRPFY	1290.47
1400.3±1.79	676-687	IDPLGLPDRPFY	1403.63
1548.0±1.37	675-687	FIDPLGLPDRPFY	1550.80
1619.5±1.51	674-687**	AFIDPLGLPDRPFY	1621.88
1775.48±1.32	673-687*	RAFIDPLGLPDRPFY	1778.07
2440.2±1.3	653-672	FDKSNPIVLRMMNDQLMFLE	2442.93
1904.63±1.56	672-687*	ERAFIDPLGLPDRPFY	1907.19
2310.6±2.5	653-671	FDKSNPIVLRMMNDQLMFL	2313.82
2017.4±1.94	671-687	LERAFIDPLGLPDRPFY	2020.35
2197.43±1.78	653-670	FDKSNPIVLRMMNDQLMF	2200.66

Boldface sequence correspond to peptides predicted to bind to MHC, see Table 7.

* On the basis of mass alone this peak could equally well be assigned to a peptide beginning at 654, however proteasomal removal of just the N-terminal amino acid is considered unlikely. If the issue were important it could be resolved by N-terminal sequencing.

** On the basis of mass alone these peaks could have been assigned to internal fragments, but given the overall pattern of digestion it was considered unlikely.

Epitope Identification

Fragments co-C-terminal with 8-10 amino acid long sequences predicted to bind HLA by the SYFPEITHI or NIH algorithms were chosen for further study. The digestion and prediction steps of the procedure can be usefully practiced in any order. Although the substrate peptide used in proteasomal digest described here was specifically designed to include predicted HLA-A2.1 binding sequences, the actual products of digestion can be checked after the fact for actual or predicted binding to other MHC molecules. Selected results are shown in Table 8.

Table 8. Predicted HLA binding by proteasomally generated fragments

VII. SEQ ID NO	VIII. PEPTIDE	HLA	SYFPEITHI	NIH
39 & (40)	(R)MMNDQLMFL	A*0201	24 (23)	1360 (722)
		A*0205	NP†	71 (42)
		A26	15	NP
		B*2705	12	50
41	RMMNDQLMF	B*2705	17	75

†No prediction

As seen in Table 8, N-terminal addition of authentic sequence to epitopes can generate still useful, even better epitopes, for the same or different MHC restriction elements. Note for example the pairing of (R)MMNDQLMFL (SEQ ID NOS. 39 and (40)) with HLA-A*02, where the 10-mer retains substantial predicted binding potential.

HLA-A*0201 binding assay

HLA-A*0201 binding studies were preformed, essentially as described in Example 1.1 above, with $PSMA_{663-671}$ (SEQ ID NO. 39) and $PSMA_{662-671}$, RMMNDQLMFL (SEQ NO. 67). As seen in figures 4, 7 and 8, this epitope exhibits significant binding at even lower concentrations than the positive control peptide (FLPSDYFPSV (HBV_{18-27}); SEQ ID NO. 108). Though not run in parallel, comparison to the controls suggests that $PSMA_{662-671}$ (which approaches the Melan A peptide in affinity) has the superior binding activity of these two PSMA peptides.

Example 2.

A multi-center clinical study is carried out using compositions as disclosed herein. The results of the study show the compositions to be useful and effective for debulking solid tumors and for generally inducing anti-angiogenic activity.

Example 3. Evaluation of a PSMA composition in the Xenotransplanted Human Vasculature Model

Generation of target antigen-reactive CTL

A.   *In vivo* immunization of mice.

HHD1 transgenic A*0201 mice (Pascolo, S., et al. *J. Exp. Med.* 185:2043-2051, 1997) were anesthetized and injected subcutaneously at the base of the tail, avoiding lateral tail veins, using 100 μl containing 100 nmol of $PSMA_{288-297}$ (SEQ ID NO. 21) and 20 μg of a HTL epitope peptide in PBS emulsified with 50 μl of IFA (incomplete Freund's adjuvant).

B.   Preparation of stimulating cells (LPS blasts).

Using spleens from 2 naïve mice for each group of immunized mice, un-immunized mice were sacrificed and their carcasses placed in alcohol. Using sterile instruments, the top dermal layer of skin on the mouse's left side (lower mid-section) was cut through, exposing the peritoneum. The peritoneum was saturated with alcohol, and the spleen aseptically extracted. The spleens were placed in a petri dish with serum-free media. Splenocytes were isolated by using sterile plungers from 3 ml syringes to mash the spleens. Cells were collected in a 50 ml conical tubes in serum-free media, rinsing dish well. Cells were centrifuged (12000 rpm, 7 min) and washed one time with RPMI. Fresh spleen cells were resuspended to a concentration of $1x10^8$ cells per ml in RPMI-10%FCS (fetal calf serum). 25g/ml lipopolysaccharide and 7 μg/ml Dextran Sulfate were added. Cell were incubated for 3 days in T-75 flasks at 37°C, with 5% CO_2. Splenic blasts were collected in 50 ml tubes pelleted (12,000 rpm, 7 min) and resuspended to $3X10^7$/ml in RPMI. The blasts were pulsed with the priming peptide at 50 μg/ml, RT 4hr. mitomycin C-treated at 25μg/ml, 37°C, 20 min and washed three times with DMEM.

C.   *In vitro* stimulation.

Three days after LPS stimulation of the blast cells and the same day as peptide loading, the primed mice were sacrificed (at 14 days post immunization) to remove spleens as above. $3x10^6$ splenocytes were co-cultured with $1x10^6$ LPS blasts/well in 24-well plates at 37°C, with 5% CO_2 in DMEM media supplemented with 10% FCS, $5x10^{-5}$ M β (beta)-mercaptoethanol, 100μg/ml streptomycin and 100 IU/ml penicillin. Cultures were fed 5% (vol/vol) ConA

supernatant on day 3 and can be transferred on day 7. An aliquot of the CTL are also tested in a standard chromium release assay to ensure activity.

Implantation and Adoptive transfer

1x10^{6} telomerase-transformed HDMEC in 10 μl of EGM-2-VM medium (Clonetics, San Diego, CA) are mixed with 0.5 ml of MATRIGEL (Becton Dickinson) on ice. The mixture is injected subcutaneously, through a 25 gauge needle, along the ventral midline of the thorax of SCID mice. One week later 1x10^{7} T cells (target epitope-reactive or sham-immunized) in 0.2 ml are injected intravenously (alternatively they can be injected intraperitoneally).

Assessment (Micromorphometry)

At one and two weeks after transfer remove implants, fix in 10% buffered overnight, embed in paraffin, and section. For immunofluorescence detection of human microvessels using anti-human type IV collagen IgG and fluorescently-labeled secondary antibody, deparifinize and retrieve antigen by microwaving thin sections 2x7 minutes in 10mM citric acid, pH 6.0. Vessel density is assessed as a function of the average number of positively stained annular structures observed in five separate, randomly selected 20x fields-of-view, from at least three sections per implant.

Example 4. A Fibronectin ED-B Vaccine in the HLA-transgenic Mouse Model

A.   Establishment of tumor

M1 tumor cells grown in complete RPMI plus 10% serum were harvested and washed with PBS by centrifugation. The cells were suspended in PBS at 5x10^{6} cells/ml and 0.5 ml of the suspension (early passage) was injected subcutaneously into the abdomen.

B.   Vaccination

A nucleotide sequence encoding an HLA-A2-restricted fibronectin ED-B domain-derived housekeeping epitope, for example ED-B$_{29-38}$ (SEQ ID NO. 103), is inserted into an appropriate vaccine vector (e.g. pVAX1 (Invitrogen Inc, Carlsbad, CA) or one of the vectors described in U.S. Patent Application No. 09/561,572 entitled "EXPRESSION VECTORS ENCODING EPITOPES OF TARGET-ASSOCIATED ANTIGENS," filed on April 28, 2001. HHD-A2 mice are injected intranodally in the inguinal lymph node with 0, 2, 10, 50, 100, and 200 μg of vector in PBS every other day over 8 days (4 injections) alternating sides for each injection (single dosage per mouse or group of mice). Injection series are started the day of tumor cell injection, at 2 weeks before, and at 4 and 10 weeks after.

C.   Evaluation

At approximately 12 weeks after injection of tumor cells visible tumors are observed in the mice receiving the vehicle instead of the vaccine. Effectiveness of the vaccine is expressed as the proportion of vaccinated animals that fail to develop a tumor in the same time frame, the relative size of tumors at the same time point, the delay in time before tumors appear in the vaccinated animals, and the dose and number of composition cycles needed to inhibit or prevent the establishment of tumor.

D.   Alternative schedule

The availability of more aggressive later passage M1 cells enables a more compressed experimental schedule. Instead mice are vaccinated on the day of tumor cell inoculation, 1 and 2 weeks before, and 3 or 4 days after injections of 1x10⁶ cells. Effectiveness of vaccination is assessed at approximately 10 days after tumor cell inoculation.

E.   Immunization with peptide

HHD-A2 mice were immunized with ED-B29-38 (SEQ ID NO. 103) in complete Freund's adjuvants and spleen cells were harvested and re-stimulated *in vitro* using standard methodology. The resulting CTL were able to specifically lyse peptide pulsed T2 cells, which are HLA-A2+ (Fig. 9).

Example 5. Epitopes and Epitope Clusters

Table 9 discloses epitopes and epitope clusters from PSMA and ED-B that can be useful in construction of compositions according to the present invention.

Table 9. SEQ ID NOS.*

SEQ ID NO	IDENTITY	SEQUENCE
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2	PSMA cDNA	Accession number: NM_004476
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4	PSMA 168-190	GMPEGDLVYVNYARTEDFFKLER
5	PSMA 169-177	MPEGDLVYV
6	PSMA 168-177	GMPEGDLVYV
7	PSMA 168-176	GMPEGDLVY
8	PSMA 167-176	QGMPEGDLVY
9	PSMA 169-176	MPEGDLVY
10	PSMA 171-179	EGDLVYVNY
11	PSMA 170-179	PEGDLVYVNY
12	PSMA 174-183	LVYVNYARTE
13	PSMA 177-185	VNYARTEDF
14	PSMA 176-185	YVNYARTEDF
15	PSMA 178-186	NYARTEDFF
16	PSMA 179-186	YARTEDFF
17	PSMA 181-189	RTEDFFKLE
18	PSMA 281-310	RGIAEAVGLPSIPVHPIGYYDAQKLLEKMG
19	PSMA 283-307	IAEAVGLPSIPVHPIGYYDAQKLLE
20	PSMA 289-297	LPSIPVHPI
21	PSMA 288-297	GLPSIPVHPI
22	PSMA 297-305	IGYYDAQKL
23	PSMA 296-305	PIGYYDAQKL
24	PSMA 291-299	SIPVHPIGY
25	PSMA 290-299	PSIPVHPIGY

26	PSMA 292-299	IPVHPIGY
27	PSMA 299-307	YYDAQKLLE
28	PSMA454-481	SSIEGNYTLRVDCTPLMYSLVHLTKEL
29	PSMA 456-464	IEGNYTLRV
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31	PSMA 457-464	EGNYTLRV
32	PSMA 461-469	TLRVDCTPL
33	PSMA 460-469	YTLRVDCTPL
34	PSMA 462-470	LRVDCTPLM
35	PSMA 463-471	RVDCTPLMY
36	PSMA 462-471	LRVDCTPLMY
37	PSMA653-687	FDKSNPIVLRMMNDQLMFLERAFIDPLGLPDRPFY
38	PSMA 660-681	VLRMMNDQLMFLERAFIDPLGL
39	PSMA 663-671	MMNDQLMFL
40	PSMA 662-671	RMMNDQLMFL
41	PSMA 662-670	RMMNDQLMF
42	PSMA 4-12	LLHETDSAV
43	PSMA 13-21	ATARRPRWL
44	PSMA 53-61	TPKHNMKAF
45	PSMA 64-73	ELKAENIKKF
46	PSMA 69-77	NIKKFLH¹NF
47	PSMA 68-77	ENIKKFLH¹NF
48	PSMA 220-228	AGAKGVILY
49	PSMA 468-477	PLMYSLVHNL
50	PSMA 469-477	LMYSLVHNL
51	PSMA 463-471	RVDCTPLMY
52	PSMA 465-473	DCTPLMYSL
53	PSMA 507-515	SGMPRISKL
54	PSMA 506-515	FSGMPRISKL
55	PSMA 211-218	GNKVKNAQ
56	PSMA 202-209	IARYGKVF
57	PSMA 217-225	AQLAGAKGV
58	PSMA 207-215	KVFRGNKVK
59	PSMA 211-219	GNKVKNAQL
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61	PSMA 268-277	LTPGYPANEY
62	PSMA 271-279	GYPANEYAY
63	PSMA 270-279	PGYPANEYAY
64	PSMA 266-274	DPLTPGYPA
65	PSMA 492-500	SLYESWTKK
66	PSMA 491-500	KSLYESWTKK
67	PSMA 486-494	EGFEGKSLY

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74	PSMA 723-732	KAWGEVKRQI
75	PSMA 723-730	KAWGEVKR
76	PSMA 722-730	SKAWGEVKR
77	PSMA 731-739	QIYVAAFTV
78	PSMA 733-741	YVAAFTVQA
79	PSMA 725-733	WGEVKRQIY
80	PSMA 727-735	EVKRQIYVA
81	PSMA 738-746	TVQAAAETL
82	PSMA 737-746	FTVQAAAETL
83	PSMA 729-737	KRQIYVAAF
84	PSMA 721-729	PSKAWGEVK
85	PSMA 723-731	KAWGEVKRQ
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87	PSMA 99-108	QWKEFGLDSV
88	PSMA 102-111	EFGLDSVELA
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90	ED-B domain of Fibronectin with flanking sequence from Fribronectin	CTFDNLSPGLEYNVSVYTVKDDKESVPISDTIIP EVPQLTDLSFVDITDSSIGLRWTPLNSSTIIGYRI TVVAAGEGIPIFEDFVDSSVGYYTVTGLEPGID YDISVITLINGGESAPTTLTQQT AVPPPTDLRFTNIGPDTMRVTW
91	ED-B domain of Fibronectin cds	Accession number: X07717
92	ED-B 4'-5	TIIPEVPQL
93	ED-B 5'-5	DTIIPEVPQL
94	ED-B 1-10	EVPQLTDLSF
95	ED-B 23-30	TPLNSSTI
96	ED-B 18-25	IGLRWTPL
97	ED-B 17-25	SIGLRWTPL
98	ED-B 25-33	LNSSTIIGY
99	ED-B 24-33	PLNSSTIIGY
100	ED-B 23-31	TPLNSSTII
101	ED-B 31-38	IGYRITVV
102	ED-B 30-38	IIGYRITVV
103	ED-B 29-38	TIIGYRITVV
104	ED-B 31-39	IGYRITVVA

105	ED-B 30-39	IIGYRITVVA
106	Melan-A 26-35$_{A>L}$	ELAGIGILTV
107	Melan-A 26-35	EAAGIGILTV
108	HBV 18-27	FLPSDYFPSV
109	HLA-B44 binder	AEMGKYSFY

¹ This H was reported as Y in the SWISSPROT database.

*Any of SEQ ID NOS. 5-17, 20-27, 29-36, 39-88, and 92-105 can be useful as epitopes in the various embodiments of the invention. Any of SEQ ID NOS. 3, 4, 18, 19, 28, 37, 38, 89 and 90 can be useful as sequences containing epitopes or epitope clusters, as described in various embodiments of the invention.

**All accession numbers used here and throughout can be accessed through the NCBI databases, for example, through the Entrez seek and retrieval system on the world wide web.

PSMA

```
LOCUS       NM_004476  2653 bp  mRNA        PRI    01-NOV-2000
DEFINITION  Homo sapiens folate hydrolase (prostate-specific membrane antigen)
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ACCESSION   NM_004476
VERSION     NM_004476.1  GI:4758397
KEYWORDS    .
SOURCE      human.
 ORGANISM   Homo sapiens
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REFERENCE   1  (bases 1 to 2653)
 AUTHORS    Israeli,R.S., Powell,C.T., Fair,W.R. and Heston,W.D.
 TITLE      Molecular cloning of a complementary DNA encoding a
            prostate-specific membrane antigen
 JOURNAL    Cancer Res. 53 (2), 227-230 (1993)
 MEDLINE    93113576
REFERENCE   2  (bases 1 to 2653)
 AUTHORS    Rinker-Schaeffer CW, Hawkins AL, Su SL, Israeli RS, Griffin CA,
            Isaacs JT and Heston WD.
 TITLE      Localization and physical mapping of the prostate-specific membrane
            antigen (PSM) gene to human chromosome 11
 JOURNAL    Genomics 30 (1), 105-108 (1995)
 MEDLINE    96129312
 PUBMED     8595888
REFERENCE   3  (bases 1 to 2653)
 AUTHORS    O'Keefe DS, Su SL, Bacich DJ, Horiguchi Y, Luo Y, Powell CT,
            Zandvliet D, Russell PJ, Molloy PL, Nowak NJ, Shows TB, Mullins C,
            Vonder Haar RA, Fair WR and Heston WD.
 TITLE      Mapping, genomic organization and promoter analysis of the human
            prostate-specific membrane antigen gene
 JOURNAL    Biochim. Biophys. Acta 1443 (1-2), 113-127 (1998)
 MEDLINE    99057588
 PUBMED     9838072
REFERENCE   4  (bases 1 to 2653)
 AUTHORS    Maraj BH, Leek JP, Karayi M, Ali M, Lench NJ and Markham AF.
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26

TITLE   Detailed genetic mapping around a putative prostate-specific
       membrane antigen locus on human chromosome 11p11.2
JOURNAL   Cytogenet. Cell Genet. 81 (1), 3-9 (1998)
MEDLINE   98358137
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ED-B domain of Fibronectin

LOCUS   HSFIBEDB   2823 bp   DNA   linear   PRI 09-AUG-1999

DEFINITION Human fibronectin gene ED-B region.

28

ACCESSION X07717

VERSION   X07717.1 GI:31406

KEYWORDS   alternate splicing; fibronectin.

SOURCE   human.

5   ORGANISM Homo sapiens

              Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;

              Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.

        REFERENCE 1 (bases 1 to 2823)

        AUTHORS   Paolella,G., Henchcliffe,C., Sebastio,G. and Baralle,F.E.

10   TITLE   Sequence analysis and *in vivo* expression show that alternative

              splicing of ED-B and ED-A regions of the human fibronectin gene are

              independent events

        JOURNAL   Nucleic Acids Res. 16 (8), 3545-3557 (1988)

        MEDLINE   88233940

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2821 ggg (SEQ ID NO. 91)

II

<u>WHAT IS CLAIMED IS</u>:

1.   A method of evaluating cell-mediated immunity comprising the steps of:

      implanting vascular cells into an immunodeficient mammal;

      establishing an immune response in the mammal; and

      assaying a characteristic to determine cell-mediated immunity in the mammal.

2.   The method of claim 1, wherein the cell-mediated immunity is directed against a neovasculature antigen.

3.   The method of claim 2, wherein the neovasculature antigen is preferentially expressed by tumor-associated neovasculature.

4.   The method of claim 2, wherein the neovasculature antigen is prostate specific membrane antigen (PSMA) or vascular endothelial growth factor receptor 2 (VEGFR2).

5.   The method of claim 1, wherein the establishing step is achieved by adoptive transfer of T-cells to the mammal.

6.   The method of claim 1, wherein the establishing step is achieved by contacting the mammal with an antigen.

7.   The method of claim 1, wherein the cell-mediated immunity is mediated by cytotoxic T lymphocytes.

8.   The method of claim 1, wherein the vascular cells are vascular endothelial cells.

9.   The method of claim 8, wherein the endothelial cells are HDMEC.

10.   The method of claim 8, wherein the endothelial cells are telomerase-transformed endothelial cells.

11.   The method of claim 1, wherein the immunodeficient mammal is a mouse.

12.   The method of claim 10, wherein the immunodeficient mouse is a SCID mouse.

13.   The method of claim 1, wherein the characterizing step comprises assessing a parameter selected from the group consisting of vessel formation, vessel destruction, vessel density, and proportion of vessels carrying blood of the host mammal.

14.   The method of claim 1, further comprising the step of implanting tumor cells or tumor tissue in the mouse.

15.   The method of claim 14, wherein the characterizing step comprises assessing a parameter selected from the group consisting of tumor presence, tumor growth, tumor size, rapidity of tumor appearance, dose of vaccine required to inhibit or prevent tumor establishment, tumor vascularization, and a proportion of necrotic tissue within the tumor.

16.   The method of claim 1, further comprising the steps of:

      providing a first population of mammals and a second populations of mammals;

      establishing cell-mediated immunity in the first population;

      differentially establishing cell-mediated immunity in the second population; and

comparing a result obtained from the first population of mammals to a result obtained from the second population of mammals.

17.   The method of claim 16, wherein the cell-mediated immunity of the first population is selected from the group consisting of naïve immunity and immunity to an irrelevant epitope.

18.   A method of evaluating cell-mediated immunity, comprising the steps of:

implanting or injecting MHC-transgenic tumor cells into an MHC-transgenic mammal;

establishing an immune response in the mammal; and

assaying a characteristic to determine cell-mediated immunity in the mammal.

19.   The method of claim 18, wherein the MHC-transgenic mammal is an HLA-transgenic mammal.

20.   The method of claim 19, wherein the HLA-transgenic mammal is an HLA-A2 transgenic mammal.

21.   The method of claim 18, wherein the mammal is a mouse.

22.   The method of claim 18, wherein the cell-mediated immunity is established by vaccination.

23.   The method of claim 22, wherein the vaccination takes place prior to, concurrent with, or subsequent to transfer of the tumor cells.

24.   The method of claim 18, wherein the cell-mediated immunity is mediated by cytotoxic T lymphocytes.

25.   The method of claim 18, wherein the neovasculature antigen is preferentially expressed by tumor-associated neovasculature.

26.   The method of claim 18, wherein the neovasculature antigen is also a tumor-associated antigen.

27.   The method of claim 26, wherein the antigen is the ED-B domain of fibronectin.

28.   The method of claim 18, wherein the characterizing step comprises assessing a parameter selected from the group consisting of tumor presence, tumor growth, tumor size, rapidity of tumor appearance, dose of vaccine required to inhibit or prevent tumor establishment, tumor vascularization, and a proportion of necrotic tissue within the tumor.

29.   The method of claim 18, further comprising the steps of:

providing a first population of mammals and a second populations of mammals;

establishing cell-mediated immunity in the first population;

differentially establishing cell-mediated immunity in the second population; and

comparing a result obtained from the first population of mammals to a result obtained from the second population of mammals.

30.   The method of claim 29, wherein the cell-mediated immunity of the first population is selected from the group consisting of naïve immunity and immunity to an irrelevant epitope.

31.   A method of treating neoplastic disease comprising the step of immunizing a mammal to induce a cellular immune response directed against an antigen differentially expressed by tumor-associated neovasculature.

32.   The method of claim 31, wherein the differentially expressed antigen is a protein.

33.   The method of claim 32, wherein the protein is prostate specific membrane antigen.

34.   The method of claim 31, wherein the antigen is the ED-B domain of fibronectin.

35.   The method of claim 32, wherein immunization is carried out with at least one peptide derived from the sequence of said protein.

36.   The method of claim 32, wherein immunization is carried out with a nucleic acid capable of conferring expression of said protein or peptides.

37.   The method of claim 36, wherein the at least one peptide comprises a housekeeping epitope.

38.   The method of claim 37, wherein the at least one peptide is co-C-terminal with the housekeeping epitope.

39.   The method of claim 37, further comprising at least one additional peptide wherein the at least one additional peptide comprises an immune epitope.

40.   The method of claim 31, comprising an additional step wherein the mammal is treated with an anti-tumor therapy active directly against cancerous cells.

41.   The method of claim 40, wherein the anti-tumor therapy is immunization against a tumor-associated antigen.

42.   The method of claim 31, wherein the cellular immune response comprises a CTL response.

43.   An immunogenic composition comprising at least one immunogen corresponding to an antigen expressed by tumor-associated neovasculature, wherein the composition can induce a cellular immune response.

44.   The composition of claim 43, wherein the immunogen is not associated with a cell conspecific with the recipient.

45.   The composition of claim 43, wherein the antigen is a protein.

46.   The composition of claim 45, wherein the protein is prostate specific membrane antigen.

47.   The composition of claim 43, wherein the antigen is the ED-B domain of fibronectin.

48.   The composition of claim 43, wherein the immunogen comprises at least one peptide.

49.   The composition of claim 43, comprising a nucleic acid capable of conferring expression of said antigen, and wherein said antigen is a protein or a peptide.

50.   The composition of claim 49, comprising at least one peptide comprising a housekeeping epitope.

51.   The composition of claim 50, wherein the at least one peptide is co-C-terminal with the housekeeping epitope.

52.   The composition of claim 50, additionally comprising at least one peptide comprising an immune epitope.

53.   The composition of claim 43, additionally comprising at least one immunogen corresponding to a tumor-associated antigen.

54.   The composition of claim 43, wherein the cellular immune response comprises a CTL response.

55.   A method of anti-tumor vaccine design comprising the steps of:

identifying an antigen differentially expressed by tumor-associated neovasculature; and

incorporating a component of the antigen into a vaccine.

56.   The method of claim 55, wherein the component is a polypeptide fragment of the antigen.

57.   The method of claim 55, wherein the component is a nucleic acid encoding the antigen or a fragment of the antigen.

58.   A method of making a research model, comprising implanting a vascular cell and a tumor cell into an immunodeficient mammal, wherein said tumor cell is implanted adjacent to said vascular cell.

59.   The method of claim 58, wherein said vascular cell is a vascular endothelial cell.

60.   The method of claim 59, wherein said vascular endothelial cell is HDMEC.

61.   The method of claim 59, wherein said vascular endothelial cell is telomerase-transformed.

62.   The method of claim 58, wherein said immunodeficient mammal is a mouse.

63.   The method of claim 62, wherein said mouse is a SCID mouse.

64.   A research model comprising an immunodeficient mammal, the mammal comprising an implanted vascular cell and an implanted tumor cell, wherein said vascular cell and said tumor cell are implanted adjacent to one another.

FIG. 1A

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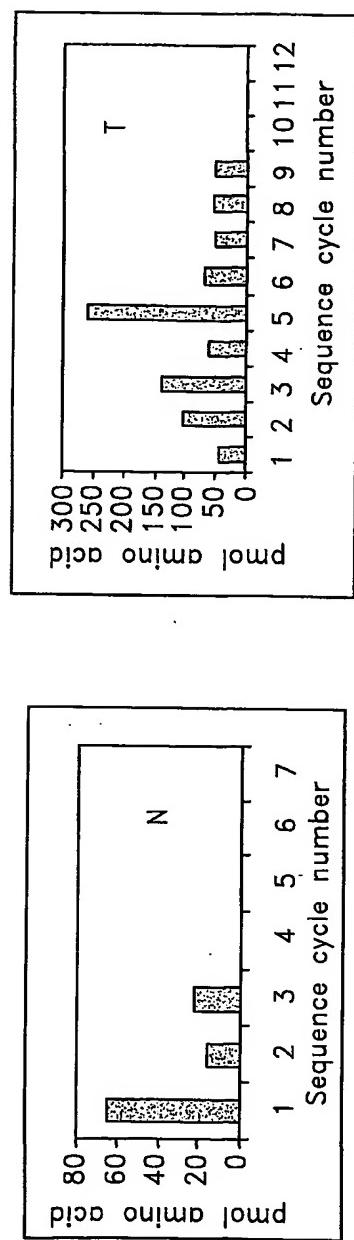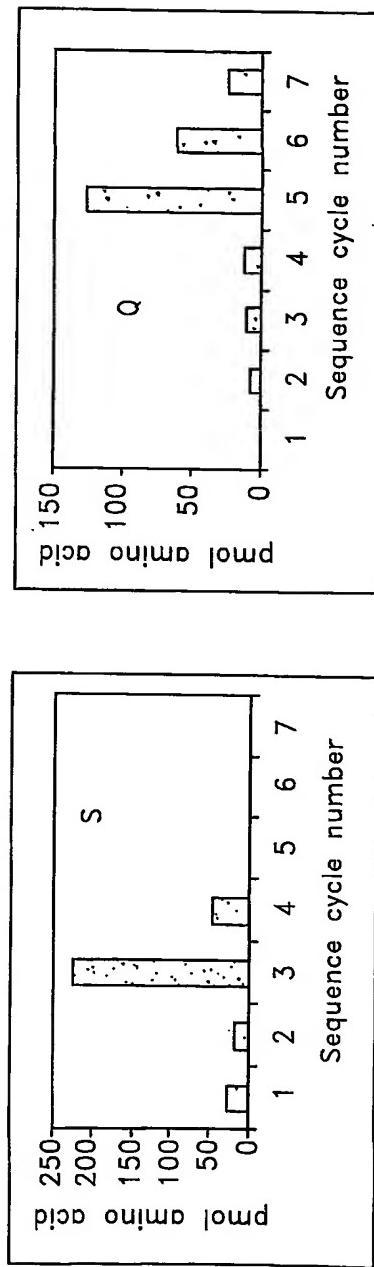
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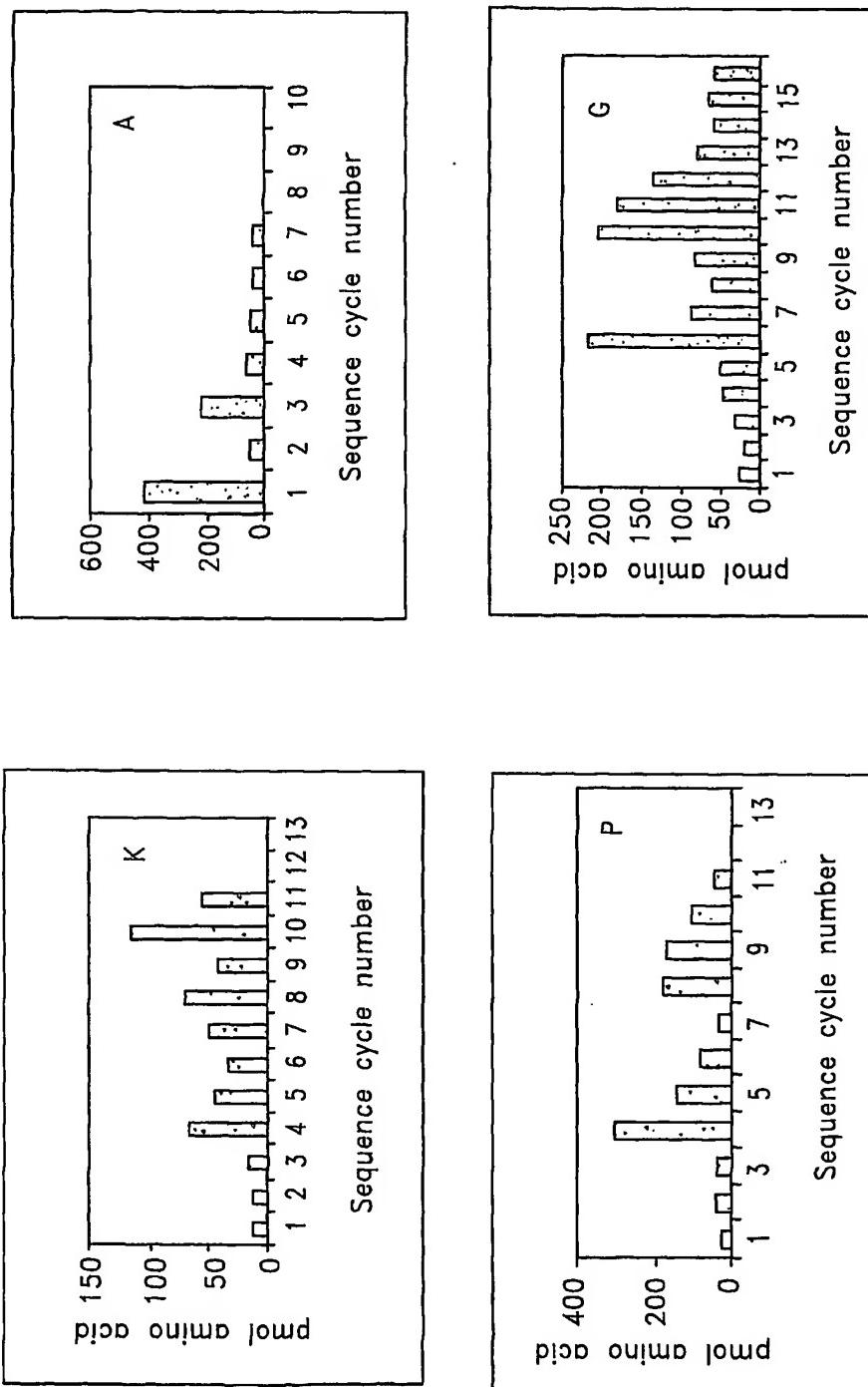
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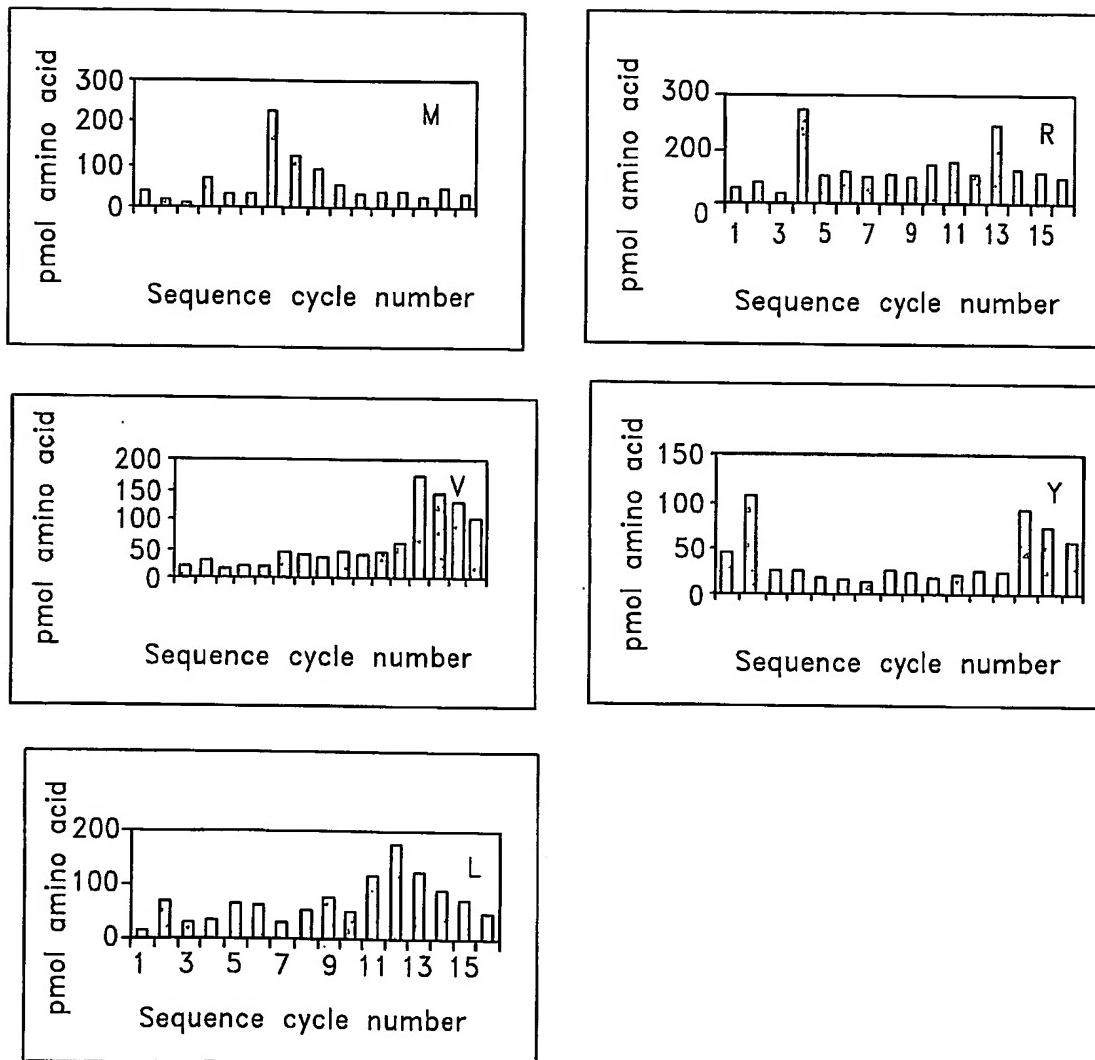
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FIG. 1C

FIG.2

HLA A2 Binding Assay

Legend:
- ---○--- HBVc 18-27 (A2)
- —□— B44
- —■— Without peptide
- ---△--- Melan A 26-35
- —●— PSMA 288-297
- —■— PSMA 168-177

X-axis: Concentration of Peptides (ug/ml): 0, 0.4, 0.8, 1.6, 3.1, 6.3, 12.5, 25, 50, 100, 200, 400

Y-axis: Mean Fluorescent Intensity: 0, 100, 200, 300, 400, 500, 600
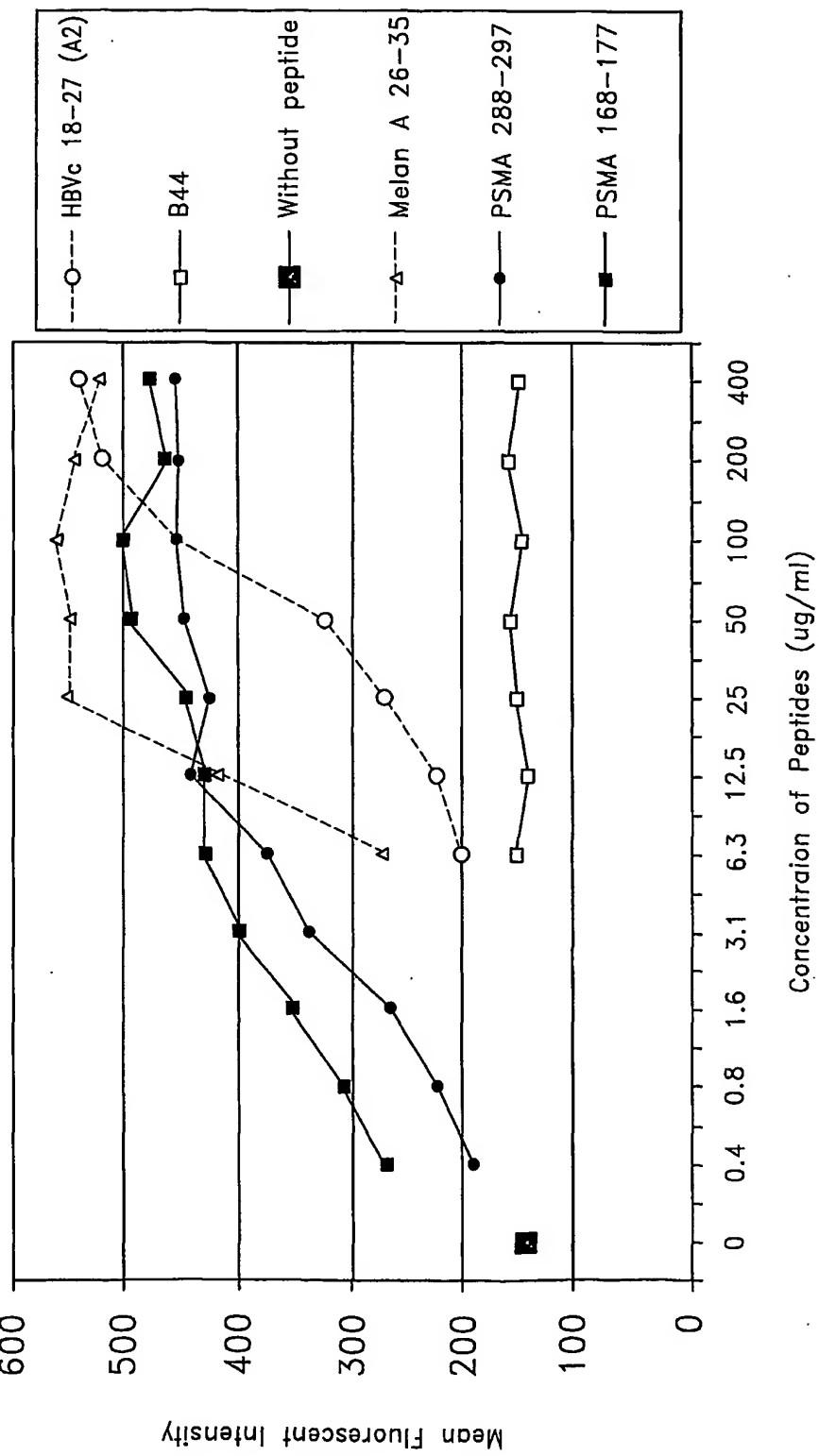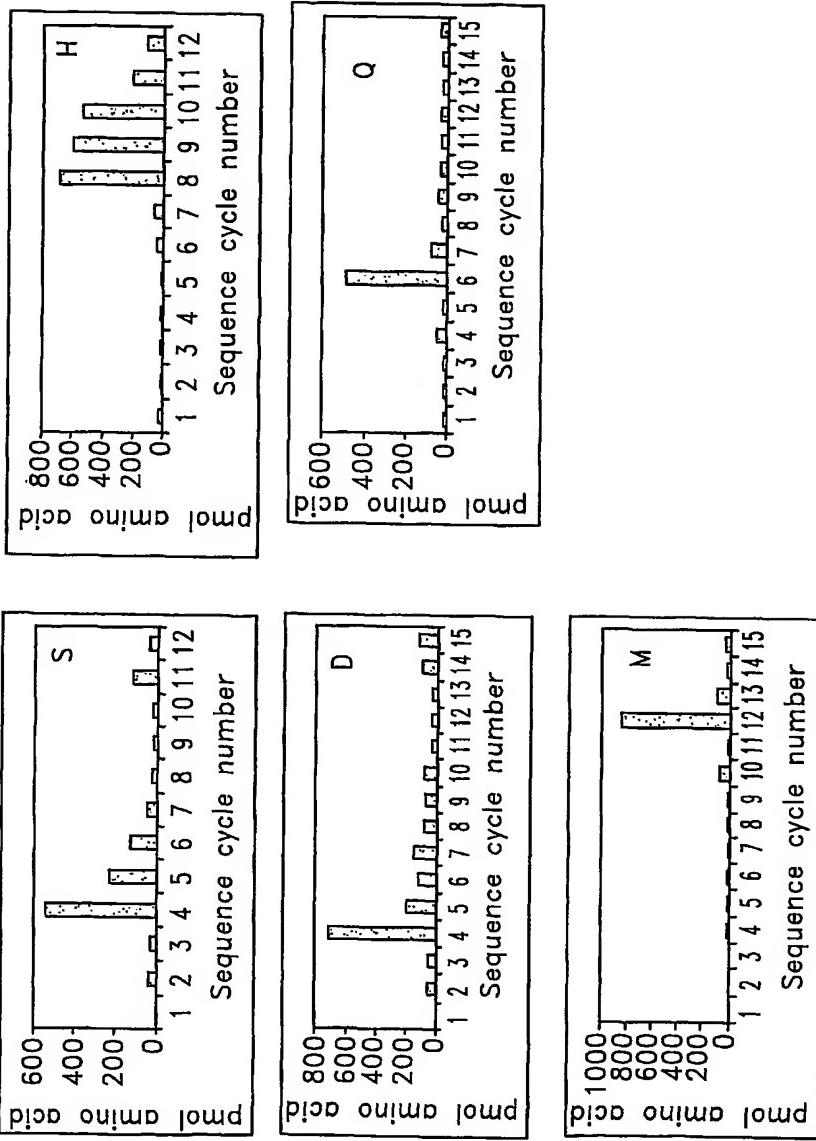
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Pool sequencing of PSMA_281_310 Digested for 60 min by Proteasome

Comparison of Peptides Binding
Affinity to HLA A2
by Binding Assay

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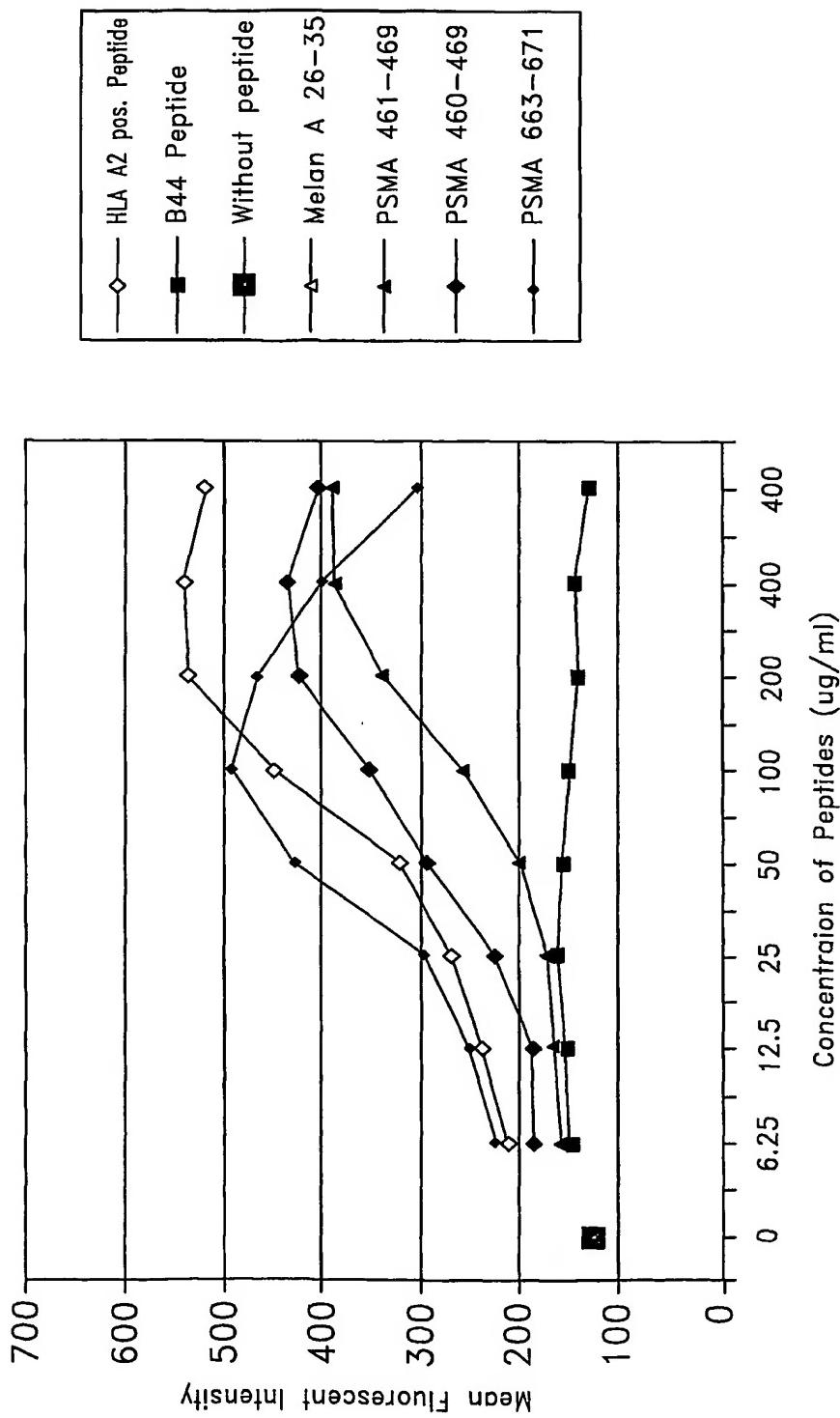
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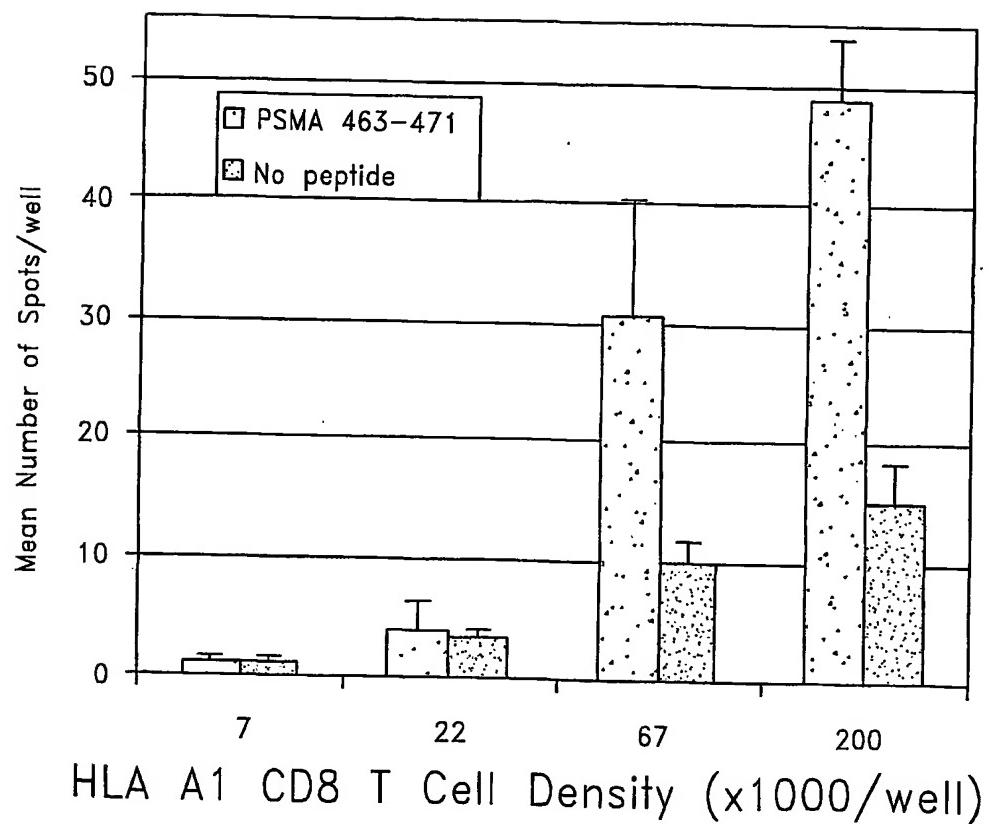
FIG. 5

Secretion of IFNgama Was Blocked by Anti-A1 Antibody
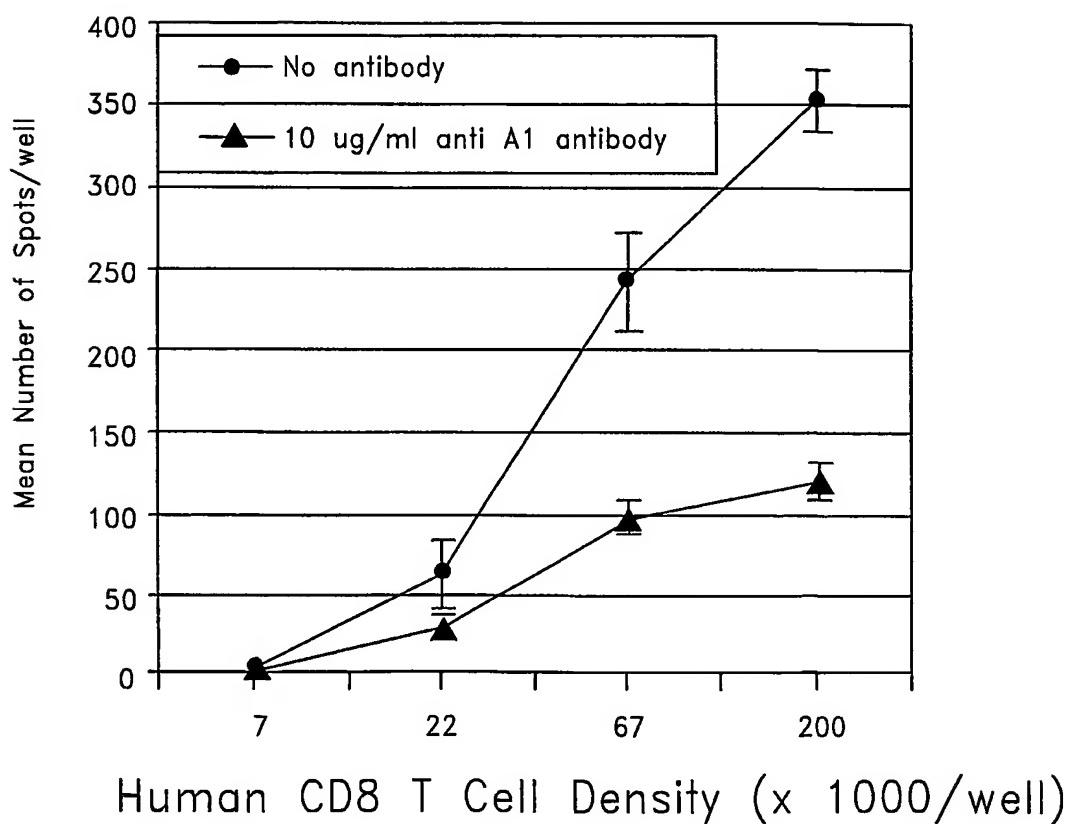
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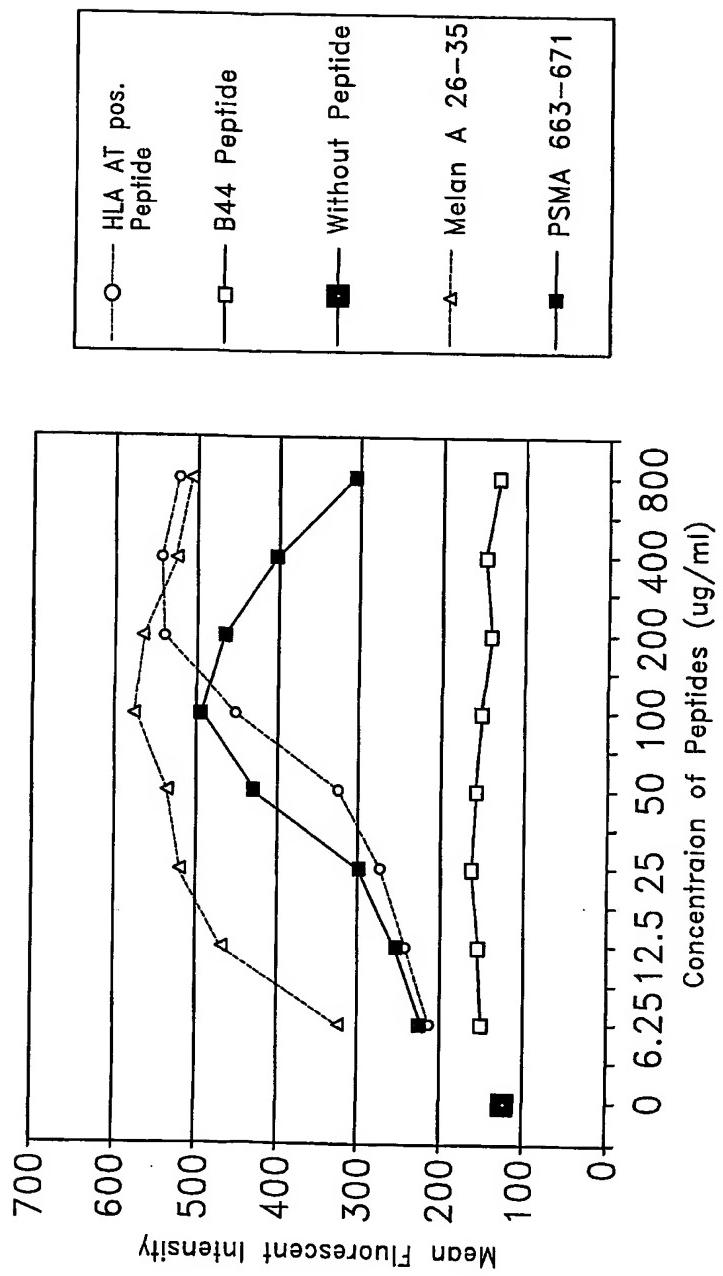
FIG. 7   Comparison of Peptides Binding Affinity to HLA A2 by Binding Assay

FIG. 8   Comparison of Peptides Binding Affinity to HLA A2 by Binding Assay
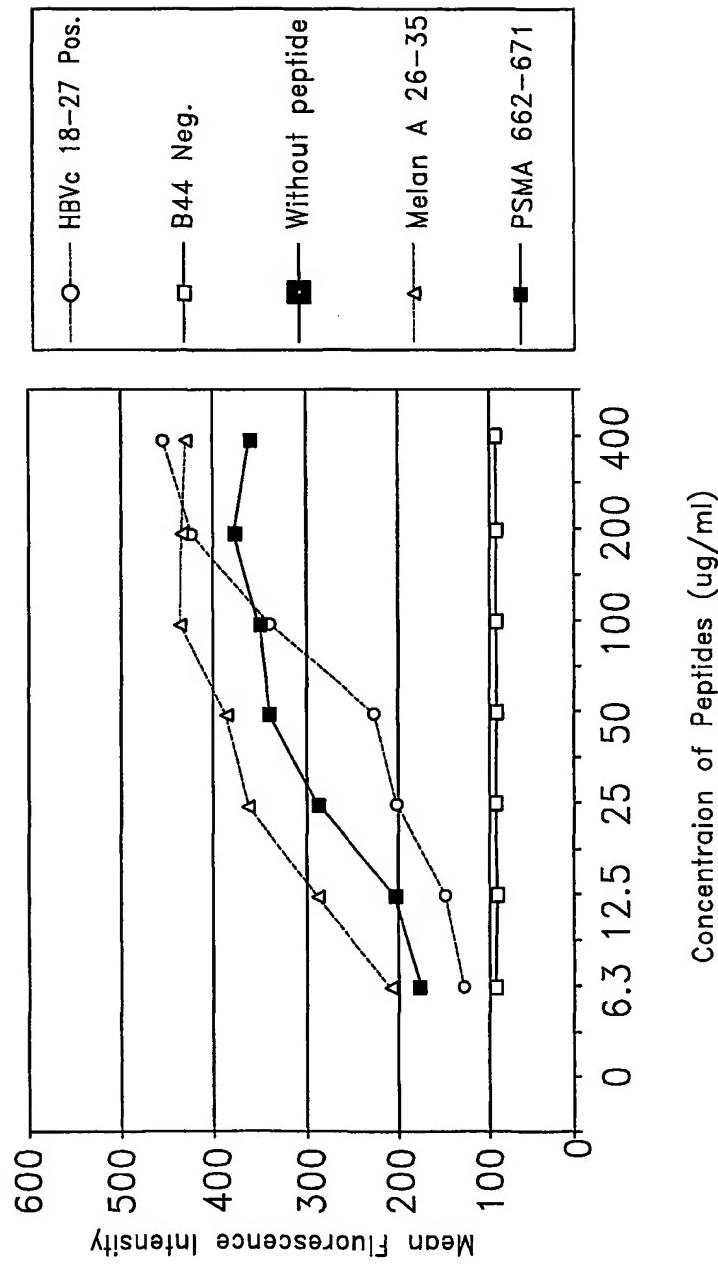
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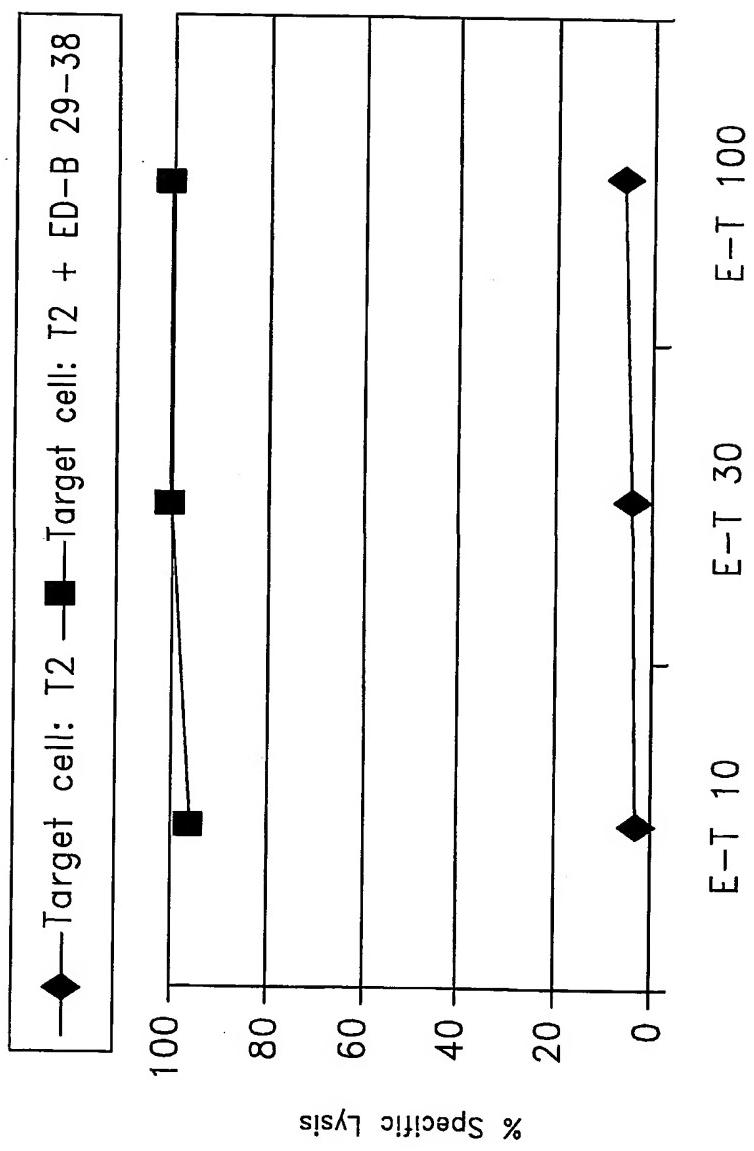
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<400> 9
Met Pro Glu Gly Asp Leu Val Tyr
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Tyr Val Asn Tyr Ala Arg Thr Glu Asp Phe
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<400> 15
Asn Tyr Ala Arg Thr Glu Asp Phe Phe
 1               5


<210> 16
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<400> 16
Tyr Ala Arg Thr Glu Asp Phe Phe
 1               5


<210> 17
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<400> 17
Arg Thr Glu Asp Phe Phe Lys Leu Glu
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<210> 18
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<212> PRT
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<213> Homo sapien

<400> 18
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Ile Gly Tyr Tyr Asp Ala Gln Lys Leu Leu Glu Lys Met Gly
            20   25   30

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<212> PRT
<213> Homo sapien

<400> 19
Ile Ala Glu Ala Val Gly Leu Pro Ser Ile Pro Val His Pro Ile Gly
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Tyr Tyr Asp Ala Gln Lys Leu Leu Glu
            20   25

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 1   5

<210> 21
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 1   5   10

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Ile Gly Tyr Tyr Asp Ala Gln Lys Leu
 1   5

<210> 23
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<400> 24
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 1   5

<210> 25
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<400> 25
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<210> 26
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Ile Pro Val His Pro Ile Gly Tyr
 1   5

<210> 27
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<400> 27
Tyr Tyr Asp Ala Gln Lys Leu Leu Glu
 1   5

<210> 28
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<400> 28
Ser Ser Ile Glu Gly Asn Tyr Thr Leu Arg Val Asp Cys Thr Pro Leu
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Met Tyr Ser Leu Val His Leu Thr Lys Glu Leu

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<210> 33
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Tyr Thr Leu Arg Val Asp Cys Thr Pro Leu
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<400> 35
Arg Val Asp Cys Thr Pro Leu Met Tyr
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<210> 36
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<400> 36
Leu Arg Val Asp Cys Thr Pro Leu Met Tyr
 1               5                   10


<210> 37
<211> 35
<212> PRT
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<400> 37
Phe Asp Lys Ser Asn Pro Ile Val Leu Arg Met Met Asn Asp Gln Leu
 1               5                   10                  15
Met Phe Leu Glu Arg Ala Phe Ile Asp Pro Leu Gly Leu Pro Asp Arg
            20                  25                  30
Pro Phe Tyr
        35


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<400> 38
Val Leu Arg Met Met Asn Asp Gln Leu Met Phe Leu Glu Arg Ala Phe
 1               5                   10                  15
Ile Asp Pro Leu Gly Leu
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<210> 39
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 1   5   10

<210> 41
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<400> 41
Arg Met Met Asn Asp Gln Leu Met Phe
 1   5

<210> 42
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Leu Leu His Glu Thr Asp Ser Ala Val
 1   5

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Ala Thr Ala Arg Arg Pro Arg Trp Leu
 1   5

<210> 44
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<400> 44

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Thr Pro Lys His Asn Met Lys Ala Phe
 1               5


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<400> 45
Glu Leu Lys Ala Glu Asn Ile Lys Lys Phe
 1               5                   10


<210> 46
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<223> Xaa = His or Tyr

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<210> 47
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<212> PRT
<213> Homo sapien

<220>
<221> VARIANT
<222> (8)...(8)
<223> Xaa = His or Tyr

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 1               5                   10


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 1               5


<210> 49
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Pro Leu Met Tyr Ser Leu Val His Asn Leu
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Leu Met Tyr Ser Leu Val His Asn Leu
 1               5


<210> 51
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Arg Val Asp Cys Thr Pro Leu Met Tyr
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<210> 52
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Asp Cys Thr Pro Leu Met Tyr Ser Leu
 1               5


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<400> 53
Ser Gly Met Pro Arg Ile Ser Lys Leu
 1               5


<210> 54
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<400> 54
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Phe Ser Gly Met Pro Arg Ile Ser Lys Leu
1               5                   10
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Gly Asn Lys Val Lys Asn Ala Gln
1               5
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```

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Ile Ala Arg Tyr Gly Lys Val Phe
1               5
```

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<211> 9
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Ala Gln Leu Ala Gly Ala Lys Gly Val
1               5
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<211> 9
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Lys Val Phe Arg Gly Asn Lys Val Lys
1               5
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Gly Asn Lys Val Lys Asn Ala Gln Leu
1               5
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<400> 61
Leu Thr Pro Gly Tyr Pro Ala Asn Glu Tyr
 1                   5                  10


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<212> PRT
<213> Homo sapien

<400> 62
Gly Tyr Pro Ala Asn Glu Tyr Ala Tyr
 1                   5


<210> 63
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<212> PRT
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<400> 63
Pro Gly Tyr Pro Ala Asn Glu Tyr Ala Tyr
 1                   5                  10


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<400> 64
Asp Pro Leu Thr Pro Gly Tyr Pro Ala
 1                   5


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 1               5


<210> 68
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<400> 68
Asp Glu Gly Phe Glu Gly Lys Ser Leu Tyr
 1               5                   10


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Thr Lys Lys Ser Pro Ser Pro Glu Phe
 1               5


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Trp Thr Lys Lys Ser Pro Ser Pro Glu Phe
 1               5                   10


<210> 71
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 1               5                   10


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Trp Gly Glu Val Lys Arg Gln Ile
 1               5


<210> 73
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Ala Trp Gly Glu Val Lys Arg Gln Ile
 1               5


<210> 74
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Lys Ala Trp Gly Glu Val Lys Arg Gln Ile
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<400> 75
Lys Ala Trp Gly Glu Val Lys Arg
 1               5


<210> 76
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<400> 76
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Gln Ile Tyr Val Ala Ala Phe Thr Val
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<400> 78
Tyr Val Ala Ala Phe Thr Val Gln Ala
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<210> 79
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Trp Gly Glu Val Lys Arg Gln Ile Tyr
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Glu Val Lys Arg Gln Ile Tyr Val Ala
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<210> 82
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1               5                   10


<210> 83
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<400> 83
Lys Arg Gln Ile Tyr Val Ala Ala Phe
1               5


<210> 84
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Pro Ser Lys Ala Trp Gly Glu Val Lys
1               5


<210> 85
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Lys Ala Trp Gly Glu Val Lys Arg Gln
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<210> 86
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Trp Lys Glu Phe Gly Leu Asp Ser Val
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<210> 87
<211> 10
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<400> 87
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Gln Trp Lys Glu Phe Gly Leu Asp Ser Val
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Glu Phe Gly Leu Asp Ser Val Glu Leu Ala
1   5   10

<210> 89
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Glu Val Pro Gln Leu Thr Asp Leu Ser Phe Val Asp Ile Thr Asp Ser
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Ser Ile Gly Leu Arg Trp Thr Pro Leu Asn Ser Ser Thr Ile Ile Gly
                20   25   30
Tyr Arg Ile Thr Val Val Ala Ala Gly Glu Gly Ile Pro Ile Phe Glu
            35   40   45
Asp Phe Val Asp Ser Ser Val Gly Tyr Tyr Thr Val Thr Gly Leu Glu
        50   55   60
Pro Gly Ile Asp Tyr Asp Ile Ser Val Ile Thr Leu Ile Asn Gly Gly
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Cys Thr Phe Asp Asn Leu Ser Pro Gly Leu Glu Tyr Asn Val Ser Val
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Tyr Thr Val Lys Asp Asp Lys Glu Ser Val Pro Ile Ser Asp Thr Ile
                20   25   30
Ile Pro Glu Val Pro Gln Leu Thr Asp Leu Ser Phe Val Asp Ile Thr
            35   40   45
Asp Ser Ser Ile Gly Leu Arg Trp Thr Pro Leu Asn Ser Ser Thr Ile
        50   55   60
Ile Gly Tyr Arg Ile Thr Val Val Ala Ala Gly Glu Gly Ile Pro Ile
65   70   75   80
Phe Glu Asp Phe Val Asp Ser Ser Val Gly Tyr Tyr Thr Val Thr Gly
                85   90   95
Leu Glu Pro Gly Ile Asp Tyr Asp Ile Ser Val Ile Thr Leu Ile Asn
                100   105   110
Gly Gly Glu Ser Ala Pro Thr Thr Leu Thr Gln Gln Thr Ala Val Pro

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Val Thr Trp
145


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ttaagggtgt caactatatt tgggatttaa ttccgatctc ccagctgcac tttccaaaac 240
caagaagtca aagcagcgat ttggacaaaa tgcttgctgt taacactgct ttactgtctg 300
tgcttcactg ggatgctgtg tgttgcagcg agtatgtaat ggagtggcag ccatggcttt 360
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  1   5   10

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Glu Val Pro Gln Leu Thr Asp Leu Ser Phe
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<210> 95
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Ser Ile Gly Leu Arg Trp Thr Pro Leu
1               5


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Leu Asn Ser Ser Thr Ile Ile Gly Tyr
1               5


<210> 99
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Pro Leu Asn Ser Ser Thr Ile Ile Gly Tyr
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<210> 102
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Ile Ile Gly Tyr Arg Ile Thr Val Val
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 1   5

<210> 105
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 1   5   10

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 1   5   10

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Glu Ala Ala Gly Ile Gly Ile Leu Thr Val
 1   5   10

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<400> 108
Phe Leu Pro Ser Asp Tyr Phe Pro Ser Val
1               5                   10


<210> 109
<211> 9
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<400> 109
Ala Glu Met Gly Lys Tyr Ser Phe Tyr
1               5
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